The Journal of Parasitology

Volume 23 FEBRUARY, 1937 Number 1

PARASITE REACTIONS TO HOST MODIFICATIONS*

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It is customary for the president of this society to make his address a discussion of some problem in which he is particularly interested. Continuing this custom, I wish to bring to your attention one method of attacking the great problem of host-parasite specificity. The term specificity as used by us does not refer to the specific identity of either host or parasite but to the specific or definite infection-relations existing between species of hosts and species of parasites. The use of the term may best be illustrated by several examples. We have found that in most cases each species of *Giardia* flagellate inhabits only one species of host and does not ordinarily live in other species of hosts in a state of nature. This we refer to as a rigid host-parasite specificity. On the other hand, the large ciliate, *Balantidium coli,* lives as a normal parasite in three species of hosts, namely, chimpanzees, pigs, and man. A condition of this sort we consider to represent a loose host-parasite specificity in which a species of parasite lives in nature in several species of hosts. Or these same relationships may be described from the standpoint of the host as follows: almost every species of host is inhabited by its own peculiar species of *Giardia*; whereas three species of hosts, pigs, chimpanzees and man, are all inhabited by the same species of *Balantidium.*

Why one species of host is in general inhabited by a group of parasites that do not occur in other species of hosts, or why one species of parasite lives in one or only a few species of hosts instead of in others that seem equally satisfactory to us, are very intriguing problems. One method of determining the factors involved is to modify hosts by subjecting them to various conditions and to note the reactions of their natural parasites or of foreign parasites introduced into them. During the past fifteen years my colleagues, students and myself have carried on

* Address of the Retiring President, American Society of Parasitologists, December 29, 1936, Atlantic City, New Jersey. Those who took part in the investigations on which this discussion is based are as follows: Justin Andrews, G. H. Boyd, L. R. Cleveland, M. S. MacDougall, Lydia Eskridge, Arnoldo Gabaldon, Robert Hegner, E. C. Nelson, H. L. Ratcliffe, Eugene Schumaker, K. S. Shah, L. G. Taliaferro, and W. H. Taliaferro.

various investigations that throw light on these problems. Our investigations have included the use of modifications of a purely laboratory type as well as alterations that might more or less easily occur in nature. The latter are of particular interest because we wish especially to know what factors account for the host-parasite specificity observed under natural conditions.

Most of the protozoa of higher animals may conveniently be separated into two groups, those that live in the digestive tract and those that live in the blood stream. The intestinal protozoa are most easily influenced by modifying the diet of the host and hence we have devoted a large part of our effort to this phase of the problem. Blood-inhabiting protozoa may also be influenced by changes in the diet of the host, since the blood carries the products of digestion to the tissues. Temperature, humidity, and muscular activity also may alter the condition of the host sufficiently to bring about visible reactions of the parasites. Drugs and sera of various sorts have been employed to change the course of infections, to eliminate parasites from the host or to vary the medium within the intestine or blood stream, but these agents could not conceivably operate in nature and our results along these lines are therefore omitted here.

With these few remarks as an introduction, I will now proceed to present a running account of our studies.

Our attention was first attracted to this subject while studying the intestinal protozoa of amphibians. Ciliates of the genus *Opalina* are commonly present in the intestine of tadpoles of various species of frogs. They persist in the adults of certain species of frogs but disappear in the green frog when the tadpoles of this species undergo metamorphosis and do not ordinarily occur in the adult green frog. Metcalf suggested that perhaps the change from the vegetable food of the tadpole to the animal food of the adult might account for the loss of the opalinds. This ex- explanation is not satisfactory since other species of frogs that retain their opalinids after metamorphosis also live on animal food as adults. So far as I know no one has yet explained why the adult green frog differs from other species of frogs with respect to the opalinid ciliates.

For several reasons the rat is more favorable than the frog for the experimental study of intestinal protozoa. We therefore transferred our attention to the rat and soon found that it was possible to modify this animal so as to influence profoundly its protozoan parasites. We noted first that certain rats that had been used by Dr. E. V. McCollum for dietary studies were free from trichomonad flagellates which are usually very abundant in the cecum. Inquiry revealed the fact that these rats had been fed throughout their lives on a diet high in animal protein. This led to experiments which gave what appeared to be conclusive results, since trichomonads disappeared from, or became much less

numerous in, rats that were fed on such a diet instead of what we call a normal diet consisting largely of carbohydrates.

Then it occurred to us that the intestinal protozoa we were supplying our students for laboratory study came principally from herbivorous animals, and a review of the literature soon revealed the fact that very few intestinal protozoa have been recorded from carnivorous animals. The evidence thus seemed quite convincing that for some reason a diet high in animal protein brings about a condition in the intestine that is unfavorable for the growth and reproduction of intestinal protozoa. This appeared to be true particularly of trichomonad flagellates which we studied more thoroughly than we did any other type.

The animal proteins that we used in our early experiments were contained in beefsteak. We next undertook to determine whether other animal proteins would have a similar effect. Rats were fed on diets containing 60 per cent of protein. The protein fed to one group of rats was derived from beefsteak, to a second from beef liver and to a third from casein. The results were quite striking. After 20 days rats fed only 20 per cent protein contained on the average about 10,000 trichomonads per cubic millimeter of cecal material, whereas rats fed on the beefsteak diet gave a count of only 1,000, those fed on the beef-liver diet contained 28,000, but those fed on the casein diet only 260. It seemed quite evident from these results that different types of protein brought about conditions within the intestine of the host sufficiently different to affect drastically the welfare of the trichomonads.

Just recently we had an opportunity to try out a diet high in vegetable protein. Groups of infected rats were fed on a normal diet, on a diet high in animal protein and on a diet high in vegetable protein, respectively. The animal protein diet contained 70 per cent of casein and the vegetable protein diet contained 77 per cent of wheat gluten. The average number of trichomonads per cubic millimeter of cecal contents was 8,270 in rats fed on a normal diet, 200 in rats fed on animal protein and 1,137 in rats fed on vegetable protein. Apparently the cecal contents are adversely affected by the vegetable protein but not as unfavorably as by the animal protein.

Two factors occurred to us that might account for the situation revealed by these experiments: first, changes in the hydrogen-ion concentration of the intestinal contents, and second, changes in the relative number of different types of bacteria. A preliminary examination of control and experimental animals indicated that no differences in hydrogen-ion concentration were present that seemed great enough to account for the results noted. Later a more careful study was made. The hydrogen-ion concentration of the cecal contents of rats fed on a normal diet was on the average about 6.78, of those fed on a diet of beefsteak

about 6.82, of those fed on a diet of beef liver about 6.42, and of those fed on a diet of casein about 7.12. Considering the wide range in the hydrogen-ion concentration of culture media in which trichomonads live successfully, it appears probable that this condition played a relatively minor role in the environmental factors responsible for the growth and multiplication of these flagellates.

Various investigators have demonstrated that profound changes occur in the bacterial flora of the rat's intestine when its diet is changed from one consisting largely of carbohydrates to one high in animal protein. We were of the opinion from the very beginning that the effects on the trichomonads were due either to changes in the types of bacteria resulting from the ingestion of large amounts of protein or to changes in the products of bacterial activity or to both these factors. An extensive series of experiments seemed to indicate a definite relation between types of bacteria and numbers of trichomonads. The character of the results is indicated in the accompanying table. It will be noted that when rats were fed on either beef or casein the number of trichomonads decreased markedly in numbers, proteolytic anaerobes increased in numbers, fermentative anaerobes increased in numbers, coli-aerogenes forms exhibited no definite trend and acidurics decreased in number.

	Av. no. trich.	Av. no. anaerobes		Av. no. aerobes	
		Proteo.	Ferment	Coli-Aerog.	Acidurics
20% protein ...	10,570	2.7	54,000	3,047	537,000
Steak, 10 days .	6,820	27.1	352,000	6,268	527,000
" 20 days .	880	37.0	361,000	8,050	188,000
20% protein ...	16,850	112.4	55,000	3,552	1,070,000
Casein, 10 days .	720	136.0	73,000	1,939	113,000
" 20 days .	260	532.0	73,000	1,174	40,000
20% protein ...	10,151	125.0	45,000	620	810,000
Liver, 10 days ..	19,800	112.0	550,000	507,730	425,000
" 20 days ..	28,000	0.4	2,440,000	1,350,000	96,000

On a liver diet the trichomonads increased in number, the proteolytic anaerobes decreased in number, the fermentative anaerobes increased in number, the coli-aerogenes forms increased in number and the acidurics decreased in number. Since the proteolytic anaerobes were low in number on a normal diet, increased in number on the steak and casein diets and decreased in number on the liver diet, the conclusion seemed justified that these bacteria were responsible for rendering the intestinal contents unfavorable for the trichomonads. There was a decrease in the number of acidurics but this likewise occurred when the favorable liver diet was fed to the rats.

The data then available indicated that on any diet the number of trichomonads varied inversely with the number of proteolytic anaerobes present.

Sometimes a condition that is unfavorable for one species of protozoon may not have any obvious effect on other species of the same genus. This led us to test the effects of a high protein diet on the human trichomonad, *T. hominis,* in rats. Our discovery that rat trichomonads can easily be eliminated by carbarsone treatment gave us trichomonad-free rats to work with. These were infected orally with *T. hominis* grown in culture. When fed on a diet high in animal protein 5 of 10 rats became trichomonad-free, and in 2 others the trichomonads were too few to detect in smears but appeared in cultures. Only one of the control rats fed on a normal diet lost its trichomonads during the experimental period of 36 days. These data indicate that when rats infected with *T. hominis* are fed on a diet high in animal protein, the intestinal contents become unfavorable for them just as they do for rat trichomonads.

Surveys that are undertaken to determine the incidence of *T. hominis* in human populations are usually unreliable. Ordinarily many infections are missed for reasons I need not discuss at present. Occasionally an infection is noted that is accompanied by a diarrhoeic condition. A meat diet has been suggested for the treatment of such cases and a number of instances have been brought to my attention in which the results were very satisfactory, both the diarrhoeic condition and the flagellates disappearing within a very few days. This suggests that a diet high in protein renders the intestinal contents of man unfavorable for *T. hominis* just as it does in rats.

I wish now to direct your attention to another method of attacking this problem using another species of intestinal protozoon. For the most part investigators have devoted their time and efforts to attempts to eradicate parasitic organisms, and comparatively few have endeavored *to improve the environment in the host in favor of the parasite.* No one knows what factors prevent a certain species of parasite from living in a given species of host, but it might be possible to modify the host in some very simple way that might even occur in nature so as to render it susceptible to infection. We have tried to modify rat hosts so that they can be infected with balantidial ciliates to which they are ordinarily resistant in nature. Our procedure was based on the results obtained in our trichomonad studies. We fed rats on diets either high in carbohydrates or high in proteins. Balantidia obtained directly from the intestine of pigs, or grown in culture or taken from experimentally infected rats were inoculated into rats either intragastrically or intracecally. Eighteen rats fed on a diet consisting of 70 per cent casein failed to become infected. Balantidia were later eliminated within 8 days from 12 rats that had been experimentally infected, by feeding them on a diet high in casein. Of 49 rats fed on a diet containing 60 per cent carbohydrates and 15 per cent casein, 47 became positive and retained the infection for

from 1 to 33 days. When the carbohydrates were increased to 85 per cent and the casein reduced to 5 per cent, 27 of 52 rats became infected and remained infected for from 3 to 44 days. A diet of 93.5 per cent carbohydrates, however, proved to be the most effective, since 89 of 101 rats became infected when this ration was used and the balantidia were retained up to 154 days. Furthermore, when rats infected on a 60 per cent or 85 per cent carbohydrate diet were changed to a 93.5 per cent carbohydrate diet the number of balantidia present increased markedly within from 5 to 8 days.

Another method of testing the influence of carbohydrates on balantidia was to inject starch in either gelatinized or granular form into the cecum of infected rats. In every case the number of balantidia increased greatly within a short time, whereas no increase in numbers occurred in the control animals. During these experiments both division and conjugation were noted among the balantidia, but no cysts were observed. The conclusion reached was that rats might become infected in nature if they were to ingest balantidia and were to subsist on a diet containing from 60 to 93.5 per cent of carbohydrates. They could not transmit the infection to other rats, however, since infective cysts apparently are not formed within the intestine of the rat.

Confirmation of these experimental results was obtained when cecal material from 79 pigs was analyzed. It was found that heavy infections with balantidia were generally accompanied by a diet high in carbohydrates in the form of grain. Large quantities of undigested carbohydrates and an intestinal flora such as would develop on a high carbohydrate diet were found in these animals and absent from pigs that were either negative or exhibited light infections with balantidia. Heavy infections with balantidia were accompanied by larger numbers of aciduric organisms and lesser numbers of lactose fermenters and proteolytic anaerobes, and light infections were accompanied by a lesser number of acidurics and a greater number of lactose fermenters and proteolytic anaerobes.

At about this stage in our investigations an observation was made that directed our attention to another phase of the subject. We were fully aware of the fact that dysentery amoebae thrive better in a medium containing starch than in media free from starch and actually ingest starch grains. We found also that trichomonads will ingest starch grains and grow and multiply best in cultures to which this carbohydrate has been added. But not until we placed starch grains in a culture containing balantidia and then watched them under a microscope did we realize how voracious a protozoon can be. These balantidia did not ingest them one by one and at infrequent intervals but swallowed many of them at a time and soon became so gorged with them as to reach twice their normal

size. Within 15 minutes 100 per cent of the balantidia had ingested starch grains.

This astonishing observation suggested to us that perhaps starch is used largely as food for the balantidia, and hence, when the host is fed on a high protein diet, these ciliates starve to death. A plan to test this hypothesis was soon developed. This included a study of the correlation between the amount of carbohydrates in the intestine and the number of species and individuals of intestinal protozoa. Guatemala was selected as the scene of our activities since the natives of tropical America are known to harbor large numbers of intestinal protozoa and to live on a diet consisting largely of carbohydrates.

We first had to devise a method of determining the presence and quantity of starch in human feces. This proved to be a real problem in itself. Then we collected 100 fecal samples from natives in Guatemala, examined them when fresh so as to record the different species present and the relative numbers of each species, and preserved a sufficient quantity of each specimen with which to determine the starch content. A large amount of time and effort was expended before we were able to evaluate our results. Unfortunately our data indicate no correlation whatever between the presence of starch and the number of species and individual protozoa in the feces, and I am inclined to believe that it will not be easy to establish such a correlation. This means that other methods will have to be devised before we can hope to explain why modifications of the host resulting from a diet high in carbohydrates favors the growth and multiplication of certain intestinal protozoa.

Various other methods of changing the intestinal contents of the host have been tried but not enough work has been done to warrant discussion except perhaps that of increasing the viscosity of the medium. Various substances were fed to rats infected with trichomonads; the most satisfactory were psyllium seed, agar-agar and gum arabic. The average number of trichomonads per unit in the cecum of rats fed on a normal diet was 392, whereas the number per unit in rats fed on psyllium seed was 55, on agar-agar, 285, and on gum arabic, 7. We also found that the number of balantidia in rats infected with ciliates from a chimpanzee was also less when a viscous condition was set up within the intestine. In normal rats there were counted 347 balantidia per centigram whereas in rats fed on agar-agar the number was 60, on linseed was 92 and on psyllium seed was 34. In no case were all of the trichomonads or all of the balantidia eliminated, but these protozoa evidently do not grow and multiply as well when the viscosity of the intestinal contents is increased by changes in the diet.

Intestinal protozoa appear to be able to withstand temperatures below zero centigrade for considerable periods of time without obvious damage,

but succumb quickly to high temperatures. This has led to various suggestions involving the use of heat as a method of eliminating these organisms from the host. For the most part temperatures high enough to destroy endoparasitic protozoa are injurious or fatal to the host, but in some cases the host survives after the death of the parasites. For example, the intestine of the large Pacific termite, *Termopsis angusticollis,* is swarming with four different types of flagellates and cannot digest the wood it uses for food without the aid of these protozoa. By subjecting these termites to a temperature of 36° C. for 24 hours all of the flagellates are destroyed, but the hosts are not harmed. This is a temperature that might occur in nature and hence experiments of this type fall within the province of this discussion. The defaunated termites continue to eat wood, but cannot digest it and therefore die in about 3 weeks in the midst of plenty. If these hosts are reinfected they are once more able to utilize the wood they eat and live indefinitely.

Temperature is not the only factor that is capable of modifying termites so as to destroy the intestinal flagellates living in them. Thus if a termite is starved for 6 days it loses one type of flagellate, *Trichonympha,* and if it is starved for 2 days longer it loses a second type of flagellate, *Leidyopsis.* Further experiments with oxygen under pressure have demonstrated that the other two types of flagellates, *Trichomonas* and *Streblomastix,* can be removed by this treatment without injury to the termites, but of course no such condition occurs in nature. However, by the use of high temperatures, starvation and oxygen under pressure, it has been shown that termites will die in about 3 weeks if all protozoa are eliminated or if all but *Streblomastix* are removed; that they will die in about 10 weeks if both *Trichonympha* and *Leidyopsis* are removed; but will live on indefinitely with any combination of flagellates if *Leidyopsis* are present or even with *Leidyopsis* alone. Thus we have proved that the symbiosis involved in this instance is between the termite and *Leidyopsis* and that the other three species are merely hitch-hikers.

Incidentally the most perfect known method of transmission of protozoa from one host to another is exhibited by termite flagellates. The young termites soon after they hatch from the egg are fed by their parents on what is known as proctodeal food. This is excreta of the adults consisting of partially-digested cellulose and many flagellates. One hundred per cent of the young termites are therefore infected soon after they emerge from the egg and are thus supplied with a complete collection of flagellates to digest their food for them.

Blood-inhabiting protozoa have also furnished material for experiments designed to test parasite reactions to host modifications. Today I wish to use the time at my disposal to discuss three methods of treating canary birds that have resulted in obvious changes in infections with the

bird malaria parasite *Plasmodium cathemerium*. The first method was to modify the sugar content of the blood by feeding the infected birds with glucose or by treating them with insulin. The clues that led to our experiments came from several sources. Bass, in 1912, found that the addition of sugar to the culture medium was necessary to cultivate malaria parasites outside of the body. In 1913, Bass and Johns reported the cultivation of the organism of human malaria in the blood of a diabetic without the addition of sugar. Certain agents that have been found to be satisfactory as provocatives in bringing on a relapse in malaria, such as epinephrin, are known to increase the sugar content of the blood. These facts suggested that the blood stream can be improved as a culture medium for the growth and multiplication of malarial parasites by the addition of sugar. According to this theory, increasing the sugar content of the blood would result in an increase in the number of parasites. *Vice versa,* the blood stream should be rendered less favorable than normal as a habitat for malarial organisms, by decreasing the sugar content. It is not necessary to give details of our experiments here. In general the data indicate that an increase in sugar content renders the blood more favorable for the malaria parasites; the number of parasites becomes greater and remains so for a period longer than normal; relapses may occur earlier in the course of the infection and more frequently than normal; and a larger proportion of the birds die as a result of the infection. When treated with insulin the parasite curve is lower than normal throughout the course of the infection. So far as I know, no attempt has been made to modify refractory foreign hosts in this way so that they may become more susceptible, but it seems to me probable that positive results would follow experiments of this nature.

Another method of modifying the host that has been used in the study of malaria is to change the atmospheric humidity and temperature. Malariologists have found that an increase in these factors favors the breeding of mosquitoes and the development of sporozoites within the mosquito, and hence is responsible, at least in part, for epidemics of malaria. We attempted to determine whether such changes modify the vertebrate host so as to effect the development of the asexual and sexual stages in the blood. For this purpose we used canary birds infected with *Plasmodium cathemerium*. In one experiment 12 birds were inoculated each with 50 milligrams of infected blood from a single host. Six were maintained under normal conditions in a room where the temperature was about 70° F. and the relative humidity about 40 per cent. The other 6 were kept in a special room at a temperature of 90° F. and a relative humidity of about 50 per cent. The course of the infections in these 12 birds was carefully followed. The data obtained show that the average number of sexual and asexual parasites was higher in the experimental

birds than in the controls and that both types of parasites were present in larger numbers in the blood for a longer period. Thus the higher temperature and humidity appeared to modify the host so that the parasites were able to set up a more severe infection extending over a longer period of time.

A second experiment was performed in which 4 birds were maintained as controls and 7 birds were subjected to an increase in temperature and relative humidity as before. The results confirmed those of the first experiment.

In a third experiment the temperature was increased to 100° F. and the relative humidity to about 90 per cent. Four experimental birds and 3 controls were used. The control birds exhibited normal infections whereas the experimental birds suffered unusually severe infections which resulted in their death.

These experiments add another method of modifying a host, such as is known to occur in nature, which has a favorable influence on the parasite.

Although we have no experimental data of our own to offer at present, it may not be out of place to call attention to the important relation between temperature and the development of malaria parasites in mosquitoes. Low temperatures retard the growth and reproduction of the plasmodia in these insects, and when the temperature reaches a certain degree development ceases entirely. The three species of *Plasmodium* that live in man differ in their reactions to low temperatures and hence the climatic temperature determines in what regions each species is capable of passing through the sexual cycle within the mosquito and consequently the geographical distribution of the different types of malaria.

Ever since the intermittent nature of malarial fever has been recognized, theories have been proposed to account for this phenomenon. Only recently have definite data on this subject been obtained as a result of experiments on birds.

Canaries infected with *P. cathemerium* were subjected to a reversed daily cycle of exposure to light and darkness. In birds kept in the darkness during the day and in the light during the night the time of sporulation changed within three days from the evening hours to the corresponding morning hours. Birds kept under normal conditions and inoculated with two groups of parasites, one sporulating in the morning and the other in the evening, did not exhibit a periodicity of 12 hours as might be expected but of 24 hours. Thus the regulatory mechanism appears to be well defined. When infected birds are exposed to the light and darkness for alternate periods of 14 hours, the periodicity changes from 24 to 28 hours, and sporulation occurs mostly at the end of the period of darkness.

Further studies indicate that light in itself is not the factor that regulates periodicity and the time of sporulation; but apparently the feeding habits of the host, which are determined by the light, are closely related to the reproductive cycle of the parasite. Just what this relation is, is still to be discovered. It is interesting to note that when monkeys (*Cebus capucinus*) infected with *P. brasilianum* were subjected to light during the night and darkness during the day for from three to six weeks, the time of sporulation changed so that within two to three weeks sporulation reached its height just after 8 P. M. instead of just after 8 A. M.

Another method of changing the character of the asexual cycle of *P. cathemerium* in canaries has been tested. Blood from infected birds was kept at 5° C. for varying lengths of time and then inoculated into fresh birds. As was to be expected, growth of the parasites at refrigerator temperature was delayed, but when inoculated into fresh canaries the asexual cycle was shortened until sporulation again occurred at the normal time of the day and a periodicity of 24 hours was regained. It is suggested that "either the asexual cycle represents a genotypic character of the parasites which can be temporarily but not permanently changed by environmental factors, or the cycle is forced on the parasites by the activities of the bird or the diurnal variations in the environment."

These experiments demonstrate what a remarkable reaction may be stimulated in a parasitic infection by modifications in the behavior of the host. However, just as in the other experiments described here, the parasitic reaction is maintained only as long as the stimulus is applied. For example, when infected birds are returned to customary day and night conditions and *Balantidium*-infected rats are returned to a diet low in carbohydrates, the host-parasite relations return to normal.

Summary. We have attempted to determine some of the factors that are responsible for host-parasite specificity among protozoa, that is, the infection in nature of a certain species of host by certain species of protozoan parasites. Our plan has been to note the reactions of natural and foreign parasites to modifications in the host. In every case the modifications were brought about by changes in the environment of the host that might easily occur in nature.

Only a beginning has been made but already a number of interesting reactions have been revealed. A carbohydrate diet renders the intestinal contents of certain species of hosts more favorable than normal for some of the intestinal protozoa natural to these hosts. A high-protein diet is correspondingly unfavorable. Of the possible factors that may play a role in these results, changes in the hydrogen-ion concentrations seem of little or no importance; the character of the bacteria resulting from these two different kinds of diets may be responsible, or these protozoa may actually require carbohydrate as a food element.

In one case, *Balantidium coli* has been successfully colonized in a foreign host, the rat. This appears to be due solely to a change in diet to one containing a higher percentage of carbohydrates than is normal for this type of host. However, no cysts are known to have been formed in the normally refractory host hence transmission to other individuals of the same species probably does not occur. We cannot, therefore, consider the rat a normal host of this ciliate until we find some factor that will stimulate the formation of cysts.

The reactions of the bird malaria parasites to the modifications cited are of such magnitude as to appear quite striking. Increasing the sugar content of the blood favors the parasite and decreasing it brings about an unfavorable condition. Increasing the temperature and relative humidity of the host's environment also brings about modifications favorable to the parasites.

Changing the periods of light and darkness are followed by definite changes in the course of infections with malaria organisms in canary birds but whether this is due to light rays, food, the muscular activity of the bird, or to some other factor is still to be determined.

At least one result of practical application has developed out of these experiments, that is, the use of a high protein diet for the elimination of certain intestinal protozoa of man. Patients suffering from digestive disturbances due to intestinal protozoa are not numerous but those who are so unfortunate as to be infected welcome with fervor the relief this type of treatment brings to them.

In conclusion, I wish to emphasize the desirability of investigations directed toward *rendering hosts more favorable as habitats* for parasitic protozoa by modifications due to known factors. By this procedure valuable data may be obtained that might not appear when efforts are aimed solely at the elimination of the organisms.

DESCRIPTION AND LIFE HISTORY OF THE TREMATODE *HAPLOMETRANA UTAHENSIS* SP. NOV. (PLAGIORCHIIDAE) FROM *RANA PRETIOSA*[1]

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For an unknown trematode found in the intestine of the western frog, *Rana pretiosa* B. & G., Lucker (1931) proposed the generic and specific names *Haplometrana intestinalis*. Later the writer discovered a second species of this genus in the same species of frogs from the Wasatch Mountains at Springville, Utah.

The frogs of this particular locality were found living in a large spring together with a rich arthropod fauna and a large number of the gastropod, *Physella utahensis* (Clench, 1925). An investigation revealed a rather high frequency of infection among the frogs and about 4 per cent of the *Physella utahensis* shedding a large Xiphidiocercaria which bore some resemblance to the adult trematode. Except for two occasions when a single specimen each of two other cercariae were found, it was the only species encountered. The only other trematode found infecting the frogs was a species of *Gorgoderina* in the urinary bladder. Since the life history of the members of *Haplometrana* was unknown, conditions appeared favorable for a study of the complete cycle.

At the outset of the investigation, it was thought that the second intermediate host of the trematode would be found among some of the animals serving as food for the frogs. This hypothesis however was discarded when the annelids, crustacea, insects, molluscs, and fish inhabiting the spring were neither infected not subject to experimental infection. The frogs alone remained as the only forms sufficiently numerous to account for the high percentage of parasitism found. Experimental infection proved them to be the second intermediate host. The cercariae encysting in the epidermis are carried away as infective metacercariae when it is shed. Frogs become infected when the cast skin containing the cysts is ingested.

It was necessary to conduct the experimental phase of the study at the University of Minnesota Zoology Laboratory at Minneapolis.

[1] The writer wishes to express appreciation to Dr. W. A. Riley for his helpful suggestions during the course of this study. Acknowledgement is also made to Dr. V. M. Tanner of Brigham Young University, Provo, Utah, and Prof. James R. Slater of Tacoma, Washington, for their kind cooperation in providing equipment and material.

MATERIAL AND METHODS

The western frog, *Rana pretiosa,* and the snail *Physella utahensis* were the principal hosts used in this study. The difficulties involved in the importation of the hosts and frog eggs from their habitats in Utah and Washington and in keeping them in captivity automatically limited the amount of material available for experimental work. Other species of frogs used were: the southern gopher frog, *Rana areolata,* obtained from a biological supply house in St. Louis, the common leopard frog, *R. pipiens,* and the green frog, *R. clamitans,* from the environs of Minneapolis.

Since preliminary studies had indicated that frogs themselves served as the second intermediate host, local species were utilized as a source of the infective stages thereby avoiding unncessary handling of the definitive host. Their feet were introduced into a small dish containing the cercariae which quickly penetrated the epidermis and encysted. Later when the skin containing the cysts was sloughed, it was fed to the definitive hosts to produce an infection.

The frogs were kept most successfully in a large aquarium containing pond water and fed laboratory raised flies of the genus *Cynomyia.* Later in the season, they were placed outside in a large tank to which flies were attracted by baits.

The cercariae used in the feeding experiments were obtained from naturally infected *Physella utahensis* collected from the same spring where the infected frogs were found to be fairly common. On two occasions laboratory raised snails were successfully infected and lived long enough to shed cercariae but susceptible frogs were not available in either case before the snails had died. These cercariae were minutely compared with those from the naturally infected snails and found to be morphologically identical; in addition they exhibited the same tropisms toward the local frogs used as the second intermediate host.

Morphological studies were made from whole mounts, both living and preserved, and sections of all stages except the free miracidia and the mother sporocysts which were not found. Since the eggs hatch in the intestine of the snail host, free individuals of the miracidia were not available. All attempts to induce hatching of the eggs by placing them in artificial digestive media, by mingling them with the dissected and crushed digestive tracts of *Physella,* and by pressing them under the coverslips were of no avail.

Whole mounts of the cercariae were made most successfully by placing them on a slide previously smeared with egg albumen and then flooding them with the fixative, according to the technique suggested by Macy (1931: 9) except that the 70 per cent alcohol was prepared

from 95 per cent stock by diluting it with corrosive acetic fluid. Whole mounts prepared from specimens killed in corrosive-acetic fixing fluid and stained in a 3 per cent aqueous solution of alum cochineal were most satisfactory. Living cercariae, metacercariae, and small maritae were studied to great advantage by first staining in a weak solution of neutral red and then mounting them in egg albumen, according to Krull (1934).

Measurements of the cercariae were made from specimens killed in hot 5 per cent formalin (Krull 1931 : 222) ; all figures given relative to the size represent the average of twenty-five specimens.

Haplometrana Lucker, 1931 emended

Generic diagnosis. *Plagiorchiinae:* Slender worms, flattened dorsoventrally, both extremities bluntly rounded. Oesophagus long and slender, extends to near acetabulum, intestinal caeca reach near to posterior end of body. Cirrus pouch moderately long, extends dorsal to and beyond posterior margin of acetabulum; seminal vesicle sac-like, occupies posterior half of cirrus pouch; cirrus protrusible; testes round, arranged tandem or obliquely. Seminal receptacle and Laurer's canal present. Uterine rami coiled transversely, pass ventral to testes, fill body width caudad from end of intestinal caeca. Vitellaria in lateral, unorganized chains which extend from near level of acetabulum posteriorly to about midway between posterior testis and hind end of body. Excretory bladder club-shaped, elongated.

Type species.—*Haplometrana intestinalis* Lucker, 1931.

Host.—*Rana pretiosa* B. & G.

Habitat.—Intestine.

Locality.—Washington, Utah.

Description of *Haplometrana utahensis* sp. nov.

Specific diagnosis.—Haplometrana: Body elongated, slender, ends rounded and slightly narrowed, sides parallel; length about 5 mm., width 0.7–0.8 mm. (fig. 1). Cuticle densely covered with spines which disappear shortly caudad to the testes, spines flattened, rectangular with ends rounded. Oral sucker subterminal, width 0.298–0.333 mm., length 0.304–0.264 mm., acetabulum in second fifth of body length, diameter 0.231–0.264 mm., opening large. Prepharynx short; pharynx with anterior margin quadrilobate, length 0.112–0.120 mm., width 0.165–0.180 mm.; oesophagus long and slender, bifurcate, arms short; intestinal caeca long, extend into posterior fifth of body length. Genital pore median, at anterior margin of acetabulum; cirrus pouch curves over and beyond posterior margin of acetabulum, length less than twice the diameter of the latter, seminal vesicle occupies basal half of cirrus pouch, is large, divided into a long posterior and a small, rounded anterior chamber separated by a constriction; testes oblique, subspherical to oval, margins normally smooth, located in middle fifth of body length, diameter less than inter-caecal space, size: anterior 0.264–0.281 mm. wide, 0.284–0.347 mm. long, posterior 0.200–0.298 mm. wide, 0.281–0.397 mm. long; well separated; vas efferens from posterior testis always passes mesad between seminal receptacle and ovary, vasa efferentia unite anterior to end of cirrus pouch to form vas deferens, latter slender throughout. Ovary to left of midline, near posterior end of second fifth of body length, spherical, oval or trigonal, width 0.264–0.314 mm., length 0.198–0.248 mm.; seminal receptacle usually oval, very close to mesocaudal border of ovary; Laurer's canal arises from the stem of the seminal receptacle; uterus extends caudad to end of body, limbs ventral to testes, in numerous transverse loops, fill body caudad from intestinal caeca. Vitellaria extend from level of ovary to middle of fourth fifth of body length or slightly

beyond, lateral except posterior to testes where follicles are frequently confluent dorsally, particularly in younger specimens, follicles embrace caeca dorsally and ventrally, transverse collecting tubules extend mesad to level of Mehlis' gland, yolk reservoir absent.

The excretory system consists of an elongated sac-like or club-shaped bladder extending anteriorly along the dorsal side of the body to the level of the caudal margin of the posterior testis (figs. 4, 9, 11). The anterior extremity is rounded and somewhat broader than the other parts. The excretory pore is terminal. The flame cell pattern is of the 2(3+3+3) (3+3+3) type. Eggs 0.020–0.024× 0.048–0.056 mm.

First intermediate hosts.—*Physella utahensis, P. ampullacea.*

Second intermediate hosts.—*Rana* spp.

Definitive host.—*Rana pretiosa.*

Habitat.—Small intestine.

Locality.—Springville, Utah.

Type specimens.—United States National Museum Helminthological Collections No. 9025; cercaria, No. 9026. Paratypes in the collection of the Division of Entomology and Economic Zoology, Univ. of Minn., and of the author.

Differential diagnosis.

Haplometrana utahensis differs from *H. intestinalis* in the following features: (1) the body is relative broader, the width being contained in the length about 5–7 times as against 8–10 times for *H. intestinalis*; (2) the acetabulum is normal, i.e., somewhat like an inverted bowl and flattened dorsally, while in *H. intestinalis* it shows a distinct suctorial disc and a dorsal, conical, muscular mass separated by a deep constriction; (3) the seminal vesicle is divided into a long posterior and a small, round anterior chamber separated by a constriction; such a structure is not given for *H. intestinalis* and was not visible in the type material studied: (4) the diameter of the testes is less than the inter-caecal space and they are always obliquely placed while in *H. intestinalis* they fill the inter-caecal space, often overlapping the caeca and are tandem; (5) the vas efferens from the posterior testis always passes mesad between the ovary and the seminal receptacle while in *H. intestinalis* it passes caudad of the seminal receptacle; (6) the vas deferens is without an enlargement which is present in *H. intestinalis*; (7) Laurer's canal arises from the stem of the seminal receptacle, whereas in *H. intestinalis* it arises directly from the body of it, and (8) there is no yolk reservoir present on the transverse yolk duct as in *H. intestinalis.*

The Life History of *Haplometrana utahensis*

After having determined the identity of the cercaria, its host, and general course of the life history, the following experiments were conducted in order to accurately determine the biological and the morphological details of the life cycle: (1) to ascertain the percentage of natural infection in wild frogs and compare it with that of experimentally infected wild frogs to determine if a significant difference might be ob-

tained; (2) by experimental infection of frogs of different species from localities well beyond the range of *Rana pretiosa,* and (3) by using cercariae from laboratory raised and infected snails to infect laboratory raised *Rana pretiosa.*

Since the eggs of *Rana pretiosa* were not available at the outset of the study, the preceding experiments were planned and executed in the order given, the first two being completed before the final one was begun. By the time it was possible to secure the frog eggs and bring the tadpoles to a usable size the laboratory raised and infected snails had died thus making it necessary to use cercariae from naturally infected snails. These cercariae were readily and positively identified as those of *H. utahensis* from any other found in the locality.

Experimental evidence.

1. Percentage of infection. All wild frogs used in the feeding experiments were held in the laboratory for a period of about three weeks before being fed infective cysts in order to allow any young specimens already established in the intestine time to develop to a size that would readily distinguish them from the younger specimens resulting from the induced infections.

A total of eighty-one wild frogs from the same habitat was examined and twenty-five individuals, or 30.6 per cent of them, were found to be naturally infected. Of the thirty-six experimentally infected wild frogs, twenty-seven individuals, or 75 per cent of the total number, were infected, an increase of 44.14 per cent over that found in frogs living in their natural habitat.

2. Experimental infection of other species of *Rana.* Tadpoles, recently metamorphosed frogs, and adults of both *Rana pipiens* and *R. clamitans* fed infective metacercariae were found to be negative in all cases when examined.

Of five large *Rana areolata* fed infective metacercariae, four were negative but the fifth, dead three weeks after feeding, was found to contain several small specimens of *Haplometrana utahensis.*

3. Experiments with laboratory raised *Rana pretiosa.* Eggs of *R. pretiosa* obtained from Paradise Valley, Washington, were kept in glass aquaria in the laboratory where they hatched and developed. At the time all four legs had appeared, ten of the young frogs were induced to eat metacercariae resulting in an infection of 80 per cent of them.

In addition to having positively identified the cercaria as that of *Haplometrana utahensis,* the above experiments established the life history and provided detailed information relative to the biology of the trematode.

General course of the life history.

The trematodes inhabit the small intestine of the frog, where the ova are deposited and carried to the outside with the feces. While the ova contain fully developed miracidia when deposited, they hatch only in the digestive tract of the snail host. The miracidia penetrate the intestinal wall in order to reach the surrounding body tissues where they develop. The cercariae, which develop in daughter sporocysts, escape from the snail and penetrate the epidermis of the frog, where they encyst. Later when the sloughed skin containing the cysts is eaten by the tadpoles or frogs, the metacercariae are freed in the intestine where they develop, thus completing the life cyclé.

Biology of the Life History Stages of *Haplometrana utahensis*

The egg and miracidium.

Although the eggs contain fully developed miracidia when deposited, they will not hatch in the open. Eggs kept in a shallow dish at room temperature for a period of forty days remained viable but unhatched until eaten by laboratory raised specimens of *Physella utahensis,* two of which lived to shed cercariae. An examination of the fecal masses revealed only the shells of hatched eggs, no miracidia being voided. Eggs exposed to water for a period of time hatched more readily when eaten by snails than did those which were ingested immediately following oviposition.

Snails fed viable eggs were later killed at intervals and sectioned in order to obtain information relative to hatching and the movements of the miracidia. Sections of specimens killed as soon as seventy-five minutes after feeding showed miracidia escaping from the eggs but none were seen in the intestinal wall or outside it.

The mother sporocyst.

The mother sporocysts were not found. Inasmuch as the eggs hatch in the digestive tract of the snails and the miracidia enter the body tissues directly, it is probable they would be found along the digestive tract as Talbot (1935: 250) found for *Lechriorchis primus* Stafford, 1905, or in the digestive gland as reported by Byrd (1935: 199) for *Renifer aniärum* (Leidy, 1891) and *Dasymetra villicaeca* Byrd, 1935.

The daughter sporocyst.

Examinations of snails a month after infection showed the oldest daughter sporocysts to be near the intestine while the younger ones were further from it. This condition indicated that the mother sporocysts establish themselves very near the intestine and the first daughter sporocysts migrate only a short distance while those individuals of the same

generation appearing later and those of succeeding ones are under the necessity of travelling a greater distance to find conditions suitable for development. In long established infections, the liver is heavily parasitized with mature daughter sporocysts so entwined about each other and in the host tissue as to be dissected free only with the greatest difficulty.

Attempts to determine the normal period of productivity in induced infections were unsuccessful as the snail hosts did not live long in captivity. One snail, however, which bore a well established infection when received at the laboratory, maintained a constant production of cercariae for a period of sixty days, when for some unaccountable reason it died while still very productive. Snails found at autopsy to be heavily infected with sporocysts were recorded as never having shed more than 200 cercariae in a period of twenty-four hours.

The cercaria.

The first cercariae appear during the seventh week following infection of the snail host. They are shed continuously throughout the day and night with the greatest number, however, appearing during the evening. When at rest, they appear as minute balls suspended from the surface film of water, from submerged objects, and lying on the bottom of the container. Aimless swimming by vigorously lashing the tail and creeping in measuring-worm fashion are the two methods of locomotion. They display no reactions to light and may be found distributed at random in the container regardless of the manner in which it may be illuminated or shaded.

Cercariae mounted on a slide in fresh egg albumen for study remained alive for twenty-four hours and when kept in cool, well aerated water, they lived as long as forty hours.

The careful introduction of a frog's foot into a finger bowl containing resting cercariae causes them to become active individually, whereas inanimate objects fail to evoke a similar response. This ability on the part of the cercariae to detect the presence of the host is probably a chemical one.

Upon coming in contact with the frog, the cercaria quickly ceases swimming and crawls rapidly over the surface of the skin for a short time before penetrating it, an act requiring about ten minutes to complete during which the tail is usually dropped. Once within the epidermis, it forms a large passageway through which it migrates some distance before secreting a delicate cyst about itself. It is believed that the secretions of the penetration glands produce a lytic effect upon the tissues, thus aiding the cercaria in forming its tunnel. It does not enter the dermis but remains in the epidermis.

The metacercaria.

The small vesicular-like metacercariae, being enclosed in a thin membrane of epithelial tissue (fig. 5) in addition to the true cyst, are carried away with the epidermis when it is cast. This occurs at intervals of about ten days in the case of laboratory frogs.

Metacercariae were found to be infective as early as thirteen hours after encystment. Their longevity in the cast skin varies with the conditions and temperature of the water, the maximum period in fresh water at room temperature being about four to five days. Cysts were observed to pass unbroken through the stomach into the small intestine where the larval trematodes escape and develop.

The marita.

The marita were found throughout the entire length of the small intestine of the frog but more frequently in the duodenum. Occasionally in frogs found dead, they were recovered from the stomach.

When first removed from the intestine and placed into a saline solution, these handsome distomes exhibit lively and graceful movements but later as the body absorbs water and becomes swollen and cylindrical the movements become very weak. Some specimens shed a few eggs when placed in cold water, others could not be induced to do so.

Specimens collected from experimental infections 10, 26, 37, and 50 days after feeding were selected from the series of developmental stages as those being most representative of the major steps of development.

During the first ten days following excystment, very little increase in body size occurs, marked differences, however, do appear in some of the organs. The uterus, not apparent in the metacercaria, reaches caudad to the level of the anterior margin of the posterior testis; the metraterm and cirrus pouch are separated, the latter having grown in length; and the testes are larger (*cf.*, figs. 17, 19). After twenty-six days of development, the body has greatly increased in size (fig. 9). The uterus, still a solid cord of cells, extends beyond the testes; the excretory bladder and the cirrus pouch are of adult form although the latter is still very short; the testes are very much enlarged; and the seminal receptacle is present. The body is much larger at thirty-seven days of age than previously; the vitelline follicles are present (fig. 11). The cirrus pouch is of adult proportions and the seminal vesicle, now clearly visible, contains spermatozoa, while the patent uterus extends posterior to the level of the ends of the intestinal caeca. The first sexually mature specimen was found fifty days after entering the frog; it contained twelve eggs (fig. 4).

Both sexually mature and immature trematodes of various sizes were found in frogs that had just come from the winter hibernation. Judging from the slow rate of growth observed in the experimental specimens

and the fact that the flukes remain over winter in the frogs, the maximum size is probably not attained before they are two years of age.

Morphological Description of the Life History Stages

The egg and miracidium.

The yellowish brown eggs are 0.020–0.024 by 0.048–0.056 mm. in size, oval, operculate, and covered with a thin, transparent shell. They contain a fully developed miracidium when deposited (fig. 18).

Morphological studies of the miracidium were made from free living eggs and sectional intra-uterine eggs. Two elongated penetration glands, each with a terminal and medial enlargement and a sigmoid excretory duct, open through minute pores at the anterior end of the body. The contents of these glands are granular and strongly eosinophilic. A primitive digestive system lies between the anterior portion of the penetration glands. Several germ balls, usually four or five in number, were observed in the body. It was not possible to distinguish any part of the excretory system in the living or sectioned specimens.

The mother sporocyst.

Attempts to obtain mother sporocysts by feeding infective eggs to snails were unsuccessful. Owing to the limited amount of living material available, it was not possible to continue this aspect of the study to a satisfactory issue.

The daughter sporocyst.

Mature daughter sporocysts are elongated, without spines or cilia, colorless, unbranched, and with a terminal birth pore which is closed by a pair of prominent lips. The body consists of two simple sacs, a thin, delicate inner one and a thick outer one (fig. 6). A large selected specimen measured four mm. in length.

Young daughter sporocysts recovered from a snail twenty-seven days after infection have a flask-shaped body. The "neck" consists of a core of large cells covered externally by a layer of cuboidal epithelium, while the "body" forms a capacious sac of two layers of flattened epithelium. The outer thick one, continuous with the cuboidal epithelium, is separated from the thin inner layer except at the base of the "neck" where the latter is continuous with the mass of cells. Through degeneration of this cell mass, the body space is enlarged and the epithelial layers become further separated and flattened. Germ balls were present.

The cercaria.

The cercaria, a Xiphidiocercaria belonging to the group of Cercariae Ornate (Lühe, 1909: 189), resembles *Cercaria hemilophura* Cort, 1915,

and more closely *Cercaria mesotyphla* Miller, 1935. The cercariae of *Alloglossidium corti* (Lamont, 1921) and *Macroderoides typicus* (Winfield, 1929) are closely related since they develop in sporocysts and possess bag-shaped excretory bladders (McCoy, 1928: 212; McMullen, 1935: 370).

The body of the cercaria is elongated with both ends rounded, the anterior being narrower than the posterior (fig. 10). Measurements taken from the average of twenty-five specimens killed in hot 5 per cent formalin are: body length 0.434 mm., width 0.188 mm.; tail 0.516 mm. long. A dorsal fin which extends along the full length of the tail is thrown into characteristic folds when the latter is contracted or bent. The oral sucker is subterminal, its length 0.084 mm., its width 0.082 mm. The acetabulum, situated in the anterior portion of the posterior half of the body has a diameter of 0.066 mm. The prepharynx, 0.027 mm. long, is followed by a large pharynx with four distinct cephalic lobes, its length is 0.0368 mm., width 0.0045 mm. A long slender oesophagus, whose distal bifurcate end bears a small pouch on the posterior side, reaches nearly to the anterior border of the acetabulum. The patent intestinal caeca reach to the posterior extremity of the body.

The anlagen of the reproductive system except the uterus and seminal receptacle are well formed and recognizable. The cirrus pouch and metraterm are fused anteriorly; the former extends caudal to the middle of the acetabulum, the latter to its posterior margin. The small testes, composed of a few large cells, are obliquely situated a short distance posterior to the acetabulum with the caudal margin of the anterior testis at the level of the cephalic border of the posterior testis. Slender, hair-like vasa efferentia extend from the anterior portion of the testes to near the basal end of the cirrus pouch where they are no longer visible. The small ovary, located to the left of the median body line at the level of the posterior margin of the acetabulum, attaches to the large Mehlis' gland by the short, stalk-like oviduct. The metraterm, arising from the Mehlis' gland, extends anterio-mesad to the anterior margin of the acetabulum.

The penetration glands, whose secretion is granular and strongly eosinophilic, are arranged in two groups of four cells each at the level of the oesophageal bifurcation. Each cell is provided with a long flattened capillary tubule (fig. 15) that leads to the anterior margin of the oral sucker where it expands to form a storage reservoir after which it bends mesad to open near the stylet (fig. 12). The stylet, 0.024 mm. long and 0.008 mm. wide, is simply pointed and without a blade or shoulder (fig. 13).

In the living specimens, the relaxed excretory bladder is roughly sigmoid in shape with the ends greatly enlarged and connected by a short

slender portion so as to form two vesicles. When in a state of contraction or in fixed material, it assumes a slender sac- or club-shape. The excretory pore is terminal. Arising from each latero-anterior margin of the excretory bladder is a thin common collecting tubule which extends anterior and lateral to a position near the level of the testes where the anterior and posterior collecting tubules unite with it, each of which has three accessory tubules terminated by three capillaries. The position of the accessory tubules and their capillary groups are shown in figure 10. Each accessory tubule forms two principal terminal divisions: one extends directly into a single capillary, the other subdivides into two capillaries. The flame cell pattern may be expressed by the formula $2(3+3+3)+(3+3+3)$.

No indication of the vitellaria was discernible. The horizontal transverse yolk ducts were seen leading to the Mehlis' gland.

The metacercaria.

Having succeeded in entering the epithelium, the cercaria secretes an exceedingly delicate and translucent cyst about itself. At the time the frog sloughs the epidermis, the metacercaria is enclosed in two membranes, one consisting of the true cyst, the other of the epithelium. Mature cysts are oval and somewhat flattened in shape with an average size at five days after encystment of 0.228 mm. long by 0.195 mm. wide. No trace of the stylet was found in cysts in the cast epidermis. The general morphology of the metacercaria is essentially that of the cercaria (fig. 17), the principal difference being the excretory bladder which has changed from a sigmoid to a bag shape.

The Systematic Position of *Haplometrana*

Lucker (1931) established the genus *Haplometrana* and distinguished it from *Haplometra* Looss, 1899, to which he considered it closely related, primarily on the basis of the location of the uterus ventral to the testes due to the large size and tandem arrangement of the latter. McMullen (1935: 52), reasoning from Lucker's erroneous description of the excretory bladder of *Haplometrana* as being Y-shaped, considered it closely related morphologically to *Plagiorchis* Lühe, 1899, and *Eustomos* MacCallum, 1921. Therefore on the basis of the evidence provided by the life histories of *Plagiorchis* and *Eustomos,* he postulated that the cercaria of *Haplometrana* would be of the polydenous type.

The life history studies on *Haplometrana* made by the writer have shown the cercaria to be a Xiphidiocercaria of the Cercariae Ornate group which develops in unbranched sporocysts. The excretory bladder is sac- or club-shaped in all the stages of the life cycle.

McMullen (1935: 377–378) called attention to the transition in the shape of the excretory bladder in members of the family Plagiorchiidae from that of the Y-shaped one of *Plagiorchis* to the perfectly sac-shaped one in *Macroderoides* Pearse, 1924, and *Alloglossidium* Simer, 1929, with intervening stages showing a progressive shortening of the arms until they have completely disappeared. Since such a transition in form does exist, he concluded that the bladder alone cannot be considered as a family character. The only character which he found to be constant within the family ". . . so far as known, is that the life history includes Xiphidiocercariae which develop in sporocysts." He therefore concluded that the course of development and the structure of the larval stages are of primary importance in distinguishing members of the Plagiorchiidae from the other families.

The life history studies of *Macroderoides typicus* (Winfield, 1929) and *Alloglossidium corti* (Lamont, 1921) by McMullen (1935) showed them to be typical of the Plagiorchiidae; the cercariae, developing in sporocysts, possess I-shaped excretory bladders which become bag shaped in the adult. This fact led McMullen to transfer them from the family Allocreadiidae to the Plagiorchiidae. These two genera fall into the subfamily *Plagiorchiinae* as defined by Mehra (1931: 170) and together with *Haplometrana* form a closely related group quite distinct from the remaining genera of the subfamily in which the excretory bladder is Y-shaped and whose cercaria, where known, are of the polydenous type.

SUMMARY

1. A new species of the trematode genus *Haplometrana* Lucker, 1931, was found infecting the intestine of *Rana pretiosa* B. & G. of the Wasatch Mountains of Utah for which the name *H. utahensis* is proposed.

2. The life cycle has been determined experimentally. The gastropod *Physella utahensis* (Clench, 1925) is the first intermediate host and the frogs themselves the second intermediate and definitive hosts.

3. The cercariae, a Xiphiocercaria of the Cercariae Ornate group, used in the experimental work of the life history were collected from the habitat of the frogs. They were demonstrated to be those of *H. utahensis*.

4. *Haplometrana* is closely related to *Macroderoides* Pearse, 1924, and *Alloglossidium* Simer, 1929, in that it possesses a bag-shaped excretory bladder in all stages of the life cycle. In this respect, they form a compact group distinct from the other members of the subfamily as known at the present.

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Explanation of Plate I

Unless otherwise stated all drawings were made with the aid of a camera lucida.

Fig. 1. Ventral view of mature specimen of *Haplometrana utahensis* sp. nov.
Fig. 2. Portion of longitudinal section of body wall showing lateral view of spines.
Fig. 3. Dorsal view of entire spines.
Fig. 4. Fifty-day old experimental marita which has just reached sexual maturity.
Fig. 5. Section of the shed epidermis of frog showing position occupied by cyst.
Fig. 6. Mature daughter sporocyst.
Fig. 7. Horizontal section of mature adult from the ventral side showing especially the arrangement of the vasa efferentia and vas deferens.
Fig. 8. Reproductive system of sexually mature female. Free hand drawing.

Explanation of Plate II

Fig. 9. Twenty-six day old experimental marita.
Fig. 10. Mature cercaria. Composite drawing from sections, whole mounts, living material, and photomicrographs.
Fig. 11. Thirty-seven day experimental marita.
Fig. 12. Anterior ends of the ducts of the penetration glands of a cercaria; free hand drawing from living material.
Fig. 13. Stylet of mature cercaria.
Fig. 14. Section of cuticle of cercaria showing spines.
Fig. 15. Cross-section of the ducts of the penetration glands of the cercaria taken at the level of the middle of oesophagus.
Fig. 16. Cross-section of tail of cercaria showing dorsal fin.
Fig. 17. Excysted metacercaria from dorsal view.
Fig. 18. Egg containing living miracidium.
Fig. 19. Ten-day old experimental marita drawn from heavily pressed living specimen.

PLATE I

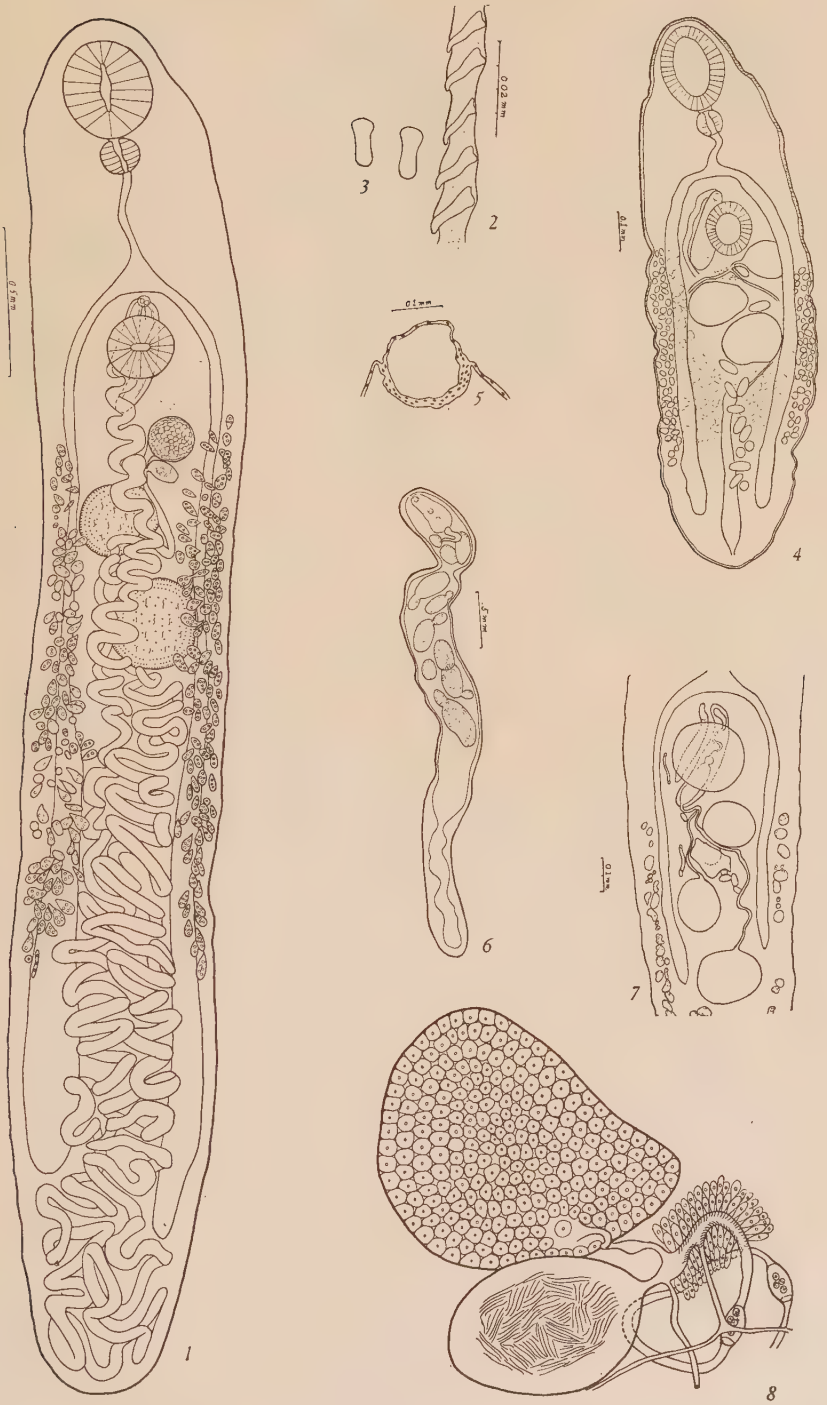
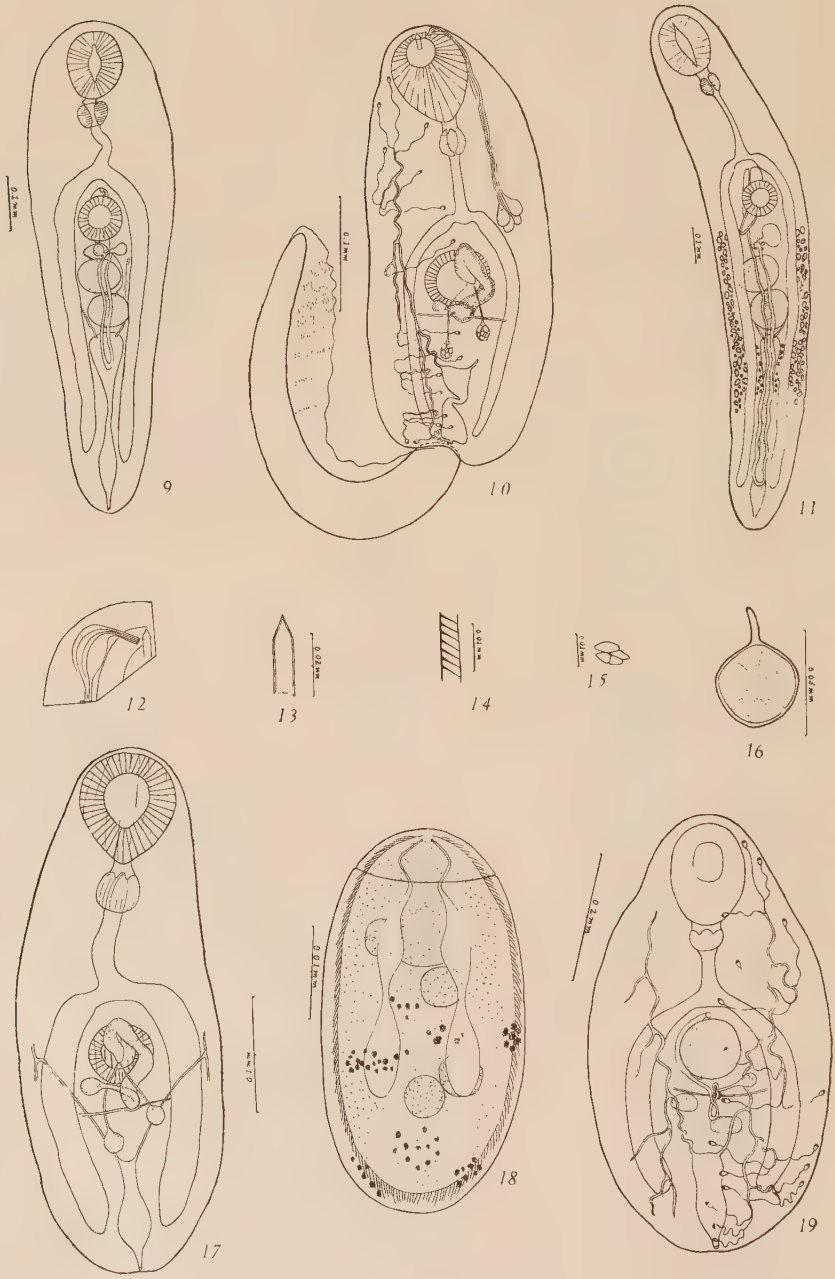


PLATE II



NEW HELMINTHS FROM NORTH CAROLINA SALAMANDERS[1]

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Our knowledge of the helminths parasitic in the salamanders of North America is very limited. Few extensive studies have been made on this group of worms. Leidy, Stafford, Walton, and Harwood have made the most important contributions. More than a thousand hosts representing nineteen species were examined for their parasites in the present study. Five new species of trematodes and two of nematodes were found.

TREMATODA

Order: Prosostomata Odhner, 1905
Sub-Order: Distomata Leuckart, 1856
Family: Gorgoderidae Looss, 1901
Sub-Family: Gorgoderinae Looss, 1899

Phyllodistomum solidum sp. nov.

Fig. 1

Description.—Length 1.82 to 2.67 mm., width 0.76 to 1.27 mm. Body not sharply divided into neck and discoidal regions. Oral sucker large, terminal, cup-shaped, 0.38 to 0.47 mm. long, 0.38 to 0.48 mm. wide; no pharynx; esophagus very short, 0.01 to 0.05 mm. in length; contraction of body often causes esophagus to assume an S-shape. Esophagus bifurcates into narrow crura which immediately expand into large muscular tubes, the anterior folds of which sometimes extend forward to side of the oral sucker. Posteriorly, they reach a distance halfway between posterior level of testes and end of body. Acetabulum larger than oral sucker, 0.44 to 0.58 mm. in length, 0.51 to 0.56 mm. in width, located between anterior and middle body thirds.

Ovary large, ovoid, and dextral, located about midway between acetabulum and posterior testis, and overlapping the right intestinal caecum; 0.04 to 0.28 mm. in length, 0.16 to 0.28 mm. in width. Vitellaria consist of a median pair of glands lying between acetabulum and ovary. They are joined by a large common vitelline duct, the whole appearing dumbbell-shaped. Each gland is about half size of ovary and has a smooth surface. A small, barely distinguished shell-gland lies at immediate median anterior border of common vitelline duct. Seminal receptacle not observed. Laurer's canal leaves oviduct posterior to vitellaria, passes sinistral and dorsal to these glands and opens on dorsal surface near margin of acetabu-

[1] This study was suggested by Dr. A. S. Pearse, Duke University. Dr. I. E. Gray, Duke University, aided in the collection and identification of hosts. Dr. F. J. Lewis, Lyons Township Junior College, compared the new phyllodistome with other species. Dr. L. G. Ingles, Chico State College, compared *Gorgoderina bilobata* n. sp. with other species and sent specimens of *G. multilobata* for comparison. Dr. Ruth Shaw Kelley, Kent State College, loaned specimens of *Diplostomulum trituri*.

lum; oviduct loops over vitelline duct and becomes an extensively coiled uterus filling whole body from median level of acetabulum to posterior end. It eventually makes its way anteriorly, dorsal to acetabulum, to open into genital sinus. Eggs small, ovoid, 30.4 by 22.4 microns.

Testes smaller than ovary and lie posterior to it in an oblique position, anterior one sinistral, posterior dextral. Vasa efferentia not observed, but vas deferens was found dorsal to the acetabulum emptying into a seminal vesicle. Weakly developed pars prostatica extends forward into an enlarged genital sinus. No cirrus found. Genital sinus communicates with outside through median genital pore which is located anterior to the acetabulum, posterior to the crural fork. A darkly staining mass of cells surrounding the anterior part of the pars prostatica may represent the prostate.

Excretory pore terminal, leading into a long slender bladder which extends unbranched to beyond the posterior testis.

Host: *Desmognathus fuscus fuscus* (Raf.).

Habitat: Urinary bladder of host.

Locality: Brook entering Nancy Rhodes Pond (Settling Pond), Durham, North Carolina.

Type Specimens: Cotypes of *Phyllodistomum solidum* have been deposited in the United States National Museum.

Discussion.—Lewis (1935), in a comparative study of all the known species of the genus Phyllodistomum, included twenty-three valid species, two of which have been found in salamanders (*P. americanum* Osborn, 1903, from *Ambystoma maculatum* (Shaw), and *P. patellare* (Sturges, 1897), from *Triturus pyrrhogaster* Boie). A new species, *P. singulare,* was described by Lynch (1936) from the salamander *Dicamptodon ensatus* (Eschscholtz). *P. solidum* is the fourth member of this genus to be reported from salamander hosts.

P. singulare is separated from all other species by the presence of well-developed stylet glands in sexually mature and seemingly full-grown worms. *P. solidum* differs from the other American species in having unlobed vitellaria, ovary, and testes, and from all but *P. pearsei* Holl, 1929, in the small size of the testes. It differs from them individually as follows: from *P. americanum* by the extra-caecal development of the uterus, large number of eggs, small size of eggs, and vitellaria smaller than ovary; from *P. carolina* Holl, 1929, by the large size and non-spatulate posterior portion; from *P. fausti* Pearse, 1924, by the small size of the ovary; from *P. lacustri* (Loewen, 1929) Lewis, 1935, by the smooth body surface and voluminous uterine folds with large numbers of eggs; from *P. pearsei* by the large size and small vitellaria; from *P. staffordi* Pearse, 1924, by the non-spatulate body and voluminous uterine folds and eggs; and from *P. superbum* Stafford, 1924, by the large size of the suckers and smooth, non-spatulate body.

P. solidum may be distinguished from the European species by the following characters: from all but *P. marinum* Laymann, 1930, *P. megalorchia* Nybelin, 1926, and *P. unicum* Odhner, 1902, in having smooth vitellaria; from all but *P. marinum, P. simile* Nybelin, 1926, and

P. unicum in having smooth ovary; from all but *P. marinum, P. pseudofolium* Nybelin, 1926, *P. simile, P. spatula* Odhner, 1902, and *P. spatulaeforme* Odhner, 1902, in having smooth testes. It differs from *P. marinum* by the non-tapering anterior end, smooth body, extra-caecal development of the uterus, and small size of the testes; and from *P. simile* by the large size, non-spatulate body, and small size of the testes.

Gorgoderina tenua sp. nov.

Fig. 2

Description.—Length 3.06 to 3.32 mm., width 0.79 to 0.85 mm.; ratio of width to length 1: 3.8. Oral sucker terminal, 0.40 to 0.42 mm. in length, 0.37 to 0.39 mm. in width; mouth terminal, opening into a short esophagus 0.04 to 0.08 mm. long; esophagus covered with small darkly-staining esophageal glands. Intestinal crura reach nearly to posterior end, for the most part concealed by uterine eggs; left branch somewhat longer. Acetabulum slightly larger than oral sucker, 0.51 to 0.57 mm. in length, 0.53 to 0.54 mm. in width; ratio of oral sucker to acetabulum 1 : 1.3; acetabulum at juncture of first and middle thirds of body, 0.34 mm. posterior to oral sucker.

Ovary sinistral, located in about mid-body length, 0.08 mm. posterior to acetabulum; spherical, sometimes pyriform, 0.26 by 0.23 mm; oviduct about mid-way between ovary and juncture of oviduct and vitelline duct; it opens on the dorsal surface in the mid-line posterior to acetabulum. Mehlis' gland situated at point of union of oviduct and vitelline duct. Uterus coils forwards, backwards, and sidewards down the left side and then up the right, passes dorsally to the acetabulum and enters the genital sinus. Genital pore large, located at bifurcation of crura. Vitellaria located between acetabulum and ovary; left gland usually dorsal to ovary; one or both glands may be lobed, with never more than four lobes; vitelline ducts join in mid-line, just anterior to ovary, forming a common vitelline duct which curves dorsally and posteriorly to enter the oviduct. Eggs small, ovoid, 29.4 by 18.0 microns.

Testes obliquely located at juncture of middle and posterior thirds of body; anterior testis dextral and smaller than posterior; it averages 0.25 by 0.24 mm., the posterior 0.38 by 0.20 mm. Distance from posterior testis to end of body, 0.88 mm. Testes somewhat irregular in shape. Vasa efferentia unite anterior to vitellaria to form the vas deferens which enters a large bulbous seminal vesicle anterior to the acetabulum. This vesicle in turn, empties into the common genital atrium.

Host: *Eurycea gutto-lineata* (Holbrook).

Habitat: Urinary bladder of host.

Locality: In brook entering Nancy Rhodes Pond (Settling Pond), Durham, North Carolina.

Type Specimens: Cotypes of *Gorgoderina tenua* have been deposited in the United States National Museum.

Discussion.—Gorgoderina tenua is more closely related to *G. multilobata* Ingles and Langston, 1933, than to any other described species. The ratios of width to length and oral sucker to acetabulum in the two species are alike. The location and shape of the testes are the same. Both have lobate vitellaria. It differs from *G. multilobata* in the following respects: very short esophagus, short distance between acetabulum and ovary, no spines around oral sucker and acetabulum, smaller size of ovary and testes, and few lobes in the vitellaria. Differences in certain body ratios are not due to contraction, for observations were made on both living and preserved material.

G. tenua differs from *G. attenuata* Stafford, 1902, by the small size of the eggs, ratio of oral sucker to acetabulum, and terminal opening of the mouth. It differs from *G. carli* Baer, 1930, *G. cedroi* and *G. cryptorchis* Travassos, 1924, *G. intermedia* Holl, 1928, *G. parvicava* Travassos, 1919, and *G. simplex* Looss, 1899, by the lobate vitellaria, small size of the eggs, and ratios between organs. It differs from *G. aurora* Ingles, 1936, by the lobate vitellaria and smaller size with corresponding smaller body ratios. Finally, it differs from *G. translucida* Stafford, 1902, by the small size of the eggs, comparatively equal size of suckers, and intra- and extra-caecal development of the uterus.

Gorgoderina bilobata sp. nov.

Fig. 3

Description.—Length 3.49 to 8.16 mm., width 0.85 to 1.72 mm.; ratio of width to length 1 : 4.2. Oral sucker terminal, 0.46 to 0.78 mm. in length, 0.43 to 0.80 mm. in width; mouth terminal, opening into a fairly long esophagus, 0.18 to 0.50 mm. long; esophagus covered with small, darkly-staining esophageal glands; intestinal crura reach nearly to posterior end, concealed largely by uterine eggs; left branch longer than right. Acetabulum somewhat larger than oral sucker, 0.50 to 1.13 mm. in length, 0.53 to 1.13 mm. in width; ratio of oral sucker to acetabulum averages 1 : 1.26. Acetabulum located just anterior to juncture of first and middle body thirds, 0.73 mm. posterior to oral sucker.

Ovary sinistral or dextral, located in about mid-body length, 0.75 mm. posterior to acetabulum; spherical, sometimes pyriform, 0.37 by 0.28 mm.; oviduct arises from dorsal side of ovary, passes anteriorly across dorsal surface to common vitelline duct, and receives this duct at point of crossing. Laurer's canal branches from oviduct about mid-way between ovary and juncture of oviduct and vitelline duct, opening on dorsal surface in mid-line anterior to vitellaria. Mehlis' gland located at point of union of oviduct and vitelline duct. Uterus coils forwards, sidewards, and backwards down right side and then up left; it widens into three or four thick loops between acetabulum and vitellaria, passes dorsally to the acetabulum and enters the genital sinus. Genital pore small, located just posterior to crural fork. Vitellaria situated anterior to ovary; both glands lobed, with never more than two lobes per gland; vitelline ducts join in mid-line and empty directly into oviduct at that point. Eggs small, ovoid, 35.6 by 22.2 microns.

Testes obliquely situated at juncture of middle and posterior body thirds; anterior somewhat sinistral and smaller than posterior, averaging 0.45 by 0.36 mm., posterior 0.54 by 0.32 mm.; irregularly oval; vasa efferentia unite anterior to vitellaria to form vas deferens which enters a large bulbous seminal vesicle anterior to acetabulum, this vesicle in turn, emptying into common genital atrium.

Host: *Ambystoma opacum* (Gravenhorst), *Desmognathus fuscus fuscus* (Raf.), *Pseudotriton montanus montanus* (Baird), *P. ruber ruber* (Sonnini).

Habitat: Urinary bladder of host.

Locality: New Hope Creek; brook entering Nancy Rhodes Pond (Settling Pond), Durham, North Carolina.

Type Specimens: Cotypes of *Gorgoderina bilobata* have been deposited in the United States National Museum.

Discussion.—Gorgoderina bilobata differs from *G. tenua* as follows: larger size with correspondingly larger body measurements, long esophagus, thick uterine folds between acetabulum and vitellaria, small genital pore, two-lobed vitellaria, and larger egg-size. It differs from *G. multilobata* Ingles and Langston, 1933, by the absence of spines

around oral sucker and acetabulum, more equal size of suckers, smaller number of vitelline lobes, fewer number of uterine folds, and larger size of eggs. It differs from *G. aurora* Ingles, 1936, by the smaller body ratios, nearly equal suckers, vitellaria fairly regularly bilobed, and larger size of eggs. It differs from *G. attenuata* Stafford, 1902, by the smaller size of the eggs, larger size of suckers, and ratios between organs. It may be distinguished from *G. carli* Baer, 1930, *G. cedroi* and *G. cryptorchis* Travassos, 1924, *G. intermedia* Holl, 1928, *G. parvicava* Travassos, 1919, and *G. simplex* Looss, 1899, by the lobate vitellaria and the ratios between organs. Finally it differs from *G. translucida* Stafford, 1902, by the smaller size of the eggs, comparatively equal size of suckers, and intra- and extra-caecal development of the uterus.

Family: Allocreadiidae Odhner, 1910
Sub-family: Allocreadiinae Odhner, 1905

Allocreadium pseudotritoni sp. nov.

Fig. 4

Description.—Length 1.98 to 2.30 mm., width 0.70 to 0.72 mm.; flattened dorso-ventrally, bluntly rounded at both ends. Cuticula smooth. Oral sucker subterminal, 0.22 to 0.25 mm. in maximum diameter; empties into a short, broad, conspicuous pharynx, 0.07 to 0.08 mm. long, 0.07 to 0.12 mm. wide; no prepharynx; esophagus short, 0.03 to 0.09 mm. long; crural fork shortly behind pharynx, well anterior to acetabulum; crura of median thickness, 0.06 to 0.08 mm., the left branch extending to anterior edge of posterior testis, the right reaching to about the middle of same testis. Acetabulum somewhat larger than oral sucker, located near juncture of anterior and middle thirds of body, 0.23 to 0.39 mm. in length, 0.27 to 0.32 mm. in width.

Ovary dextral, 0.17 to 0.22 mm. in length, 0.15 to 0.17 mm. in width; position varies from immediately behind acetabulum to a distance halfway between acetabulum and anterior testis. Oviduct arises from anterior ventral margin of ovary and passes posteriorly a short distance where it receives duct from seminal receptacle. Seminal receptacle spherical, 0.07 by 0.09 mm.; its duct winds forward and receives Laurer's canal. This latter tube winds dorsally and opens on surface just posterior to acetabulum. The combined Laurer's canal and seminal duct swing mediad to join oviduct which in turn, receives common vitelline duct in center of body; oviduct passes into cotype which is surrounded by a comparatively inconspicuous Mehlis' gland, continues into uterus which makes one or two coils between anterior testis and ovary and then extends in a more or less winding course to left and dorsal surface of acetabulum to empty at genital pore. Uterus contains few ovoid eggs with a yellowish shell, 70 to 80 microns in length, 40 to 50 microns in width. Follicular vitellaria extend from posterior margin of oral sucker to posterior end of body, filling space behind testes; they do not form a continuous band to fill space in front of acetabulum; drained by two vitelline ducts which empty into a well-defined, circular vitelline reservoir located in about mid-body region, anterior to anterior testis.

Testes in tandem arrangement within crural limits in posterior third of body, somewhat lobate, or approximately equal size, 0.22 to 0.25 mm. long, 0.26 mm. wide; vas efferens from posterior testis arises near anterior dorsal margin,

passes laterad, and continues along inner margin of intestinal caecum to acetabulum; vas efferens from anterior testis also arises from anterior dorsal margin, passes forward, swings to left, and joins its fellow at lateral margin of acetabulum to form a short vas deferens. This duct immediately expands into a seminal vesicle, continuing as a sinuous ejaculatory duct sometimes surrounded by a weakly developed pars prostatica. The whole is enveloped by a true cirrus sac, 0.25 to 0.26 mm. in length, 0.04 to 0.07 mm. in width. Distal extremity of cirrus sac empties with uterus through a small, median genital pore immediately posterior to crural fork.

Excretory bladder simple and sacculate, empties by median terminal pore.

Immature specimens were found in which vitellaria had not yet developed. Testes smaller than ovary, acetabulum considerably larger than oral sucker, cirrus sac noticeably prominent. A pair of deeply-staining eyespots occur at the lateral margins, posterior to the oral sucker.

Host: *Pseudotriton montanus montanus* (Baird), *P. ruber ruber* (Sonnini).

Habitat: Intestine of host.

Locality: Brook entering Nancy Rhodes Pond (Settling Pond), Durham, North Carolina.

Type Specimens: Cotypes of *Allocreadium pseudotritoni* have been deposited in the United States National Museum.

Discussion.—The writer planned originally to include a complete discussion of all the species of the genus Allocreadium. A review of the literature, however, indicates that much confusion exists as to the status of the various species. Such discussion would involve a consideration of many of the genera in the family Allocreadiidae, which is beyond the scope of the present paper.

As far as can be determined, only two other valid species of Allocreadium have been reported from North America, *A. lobatum* Wallin, 1910, and *A. boleosomi* Pearse, 1924. *A. pseudotritoni* is the first species of this genus to be reported from salamanders. All others were collected from fishes. *A. pseudotritoni* differs from *A. lobatum* as follows: testes not markedly lobed, vitellaria extending anterior to acetabulum, esophagus short, and seminal receptacle spherical; and from *A. boleosomi:* suckers approximately equal, uterus extending between anterior testis and acetabulum, and vitellaria extending to oral sucker.

Family: Strigeidae Railliet, 1919

Sub-Family: Polycotylinae (Viana, 1924) v. Nordmann, 1928

Diplostomulum desmognathi sp. nov.

Figs. 5, 6

Description.—Forebody ovate, widest portion anterior to acetabulum; hindbody small. Body measurements for both living and preserved specimens shown in Tables 1 and 2.

Oral sucker terminal, somewhat smaller than acetabulum; short prepharynx; short esophagus, twice length of prepharynx; crura very narrow, reaching below posterior level of holdfast organ. Acetabulum oval, located just posterior to middle of body. Cuticula armed with minute spines that are smaller on posterior portions of body. Reserve bladder system with four transverse commissural

TABLE 1.—*Measurements in Millimeters of Ten Living Diplostomulum desmognathi not Subjected to Pressure.*

	Length			Width		
	Minimum	Maximum	Average	Minimum	Maximum	Average
Body extended	0.47	0.71	0.528	0.10	0.24	0.169
" at rest	0.31	0.50	0.387	0.21	0.27	0.246
" contracted ...	0.25	0.37	0.307	0.24	0.34	0.287
Hindbody	0.04	0.08	0.058	0.07	0.09	0.077
Oral Sucker	0.04	0.05	0.042	0.04	0.05	0.043
Pharynx	0.02	0.02	0.020	0.01	0.02	0.018
Acetabulum	0.04	0.06	0.052	0.04	0.07	0.059
Holdfast Organ ...	0.07	0.08	0.073	0.06	0.09	0.074

TABLE 2.—*Measurements in Millimeters of Ten Diplostomulum desmognathi mounted in toto.*

	Length			Width		
	Minimum	Maximum	Average	Minimum	Maximum	Average
Body	0.29	0.38	0.326	0.20	0.27	0.239
Hindbody	0.01	0.02	0.018	0.05	0.07	0.060
Oral Sucker	0.02	0.04	0.035	0.02	0.04	0.029
Pharynx	0.01	0.02	0.018	0.01	0.02	0.015
Acetabulum	0.02	0.07	0.035	0.04	0.06	0.048
Holdfast Organ ...	0.05	0.07	0.058	0.06	0.08	0.068

vessels with dendritic branches; branches reach posterior end and fill space behind holdfast organ; calcareous concretions spherical with sometimes as many as three or four in each vesicle. No lateral suckers present.

Host: *Desmognathus fuscus fuscus* (Raf.), *D. phoca* (Matthes), and *D. quadramaculatus* (Holbrook).

Habitat: Body cavity of host.

Locality: Bent Creek, Pisgah National Forest, Buncombe County, North Carolina.

Type Specimens: Cotypes of *Diplostomulum desmognathi* have been deposited in the United States National Museum.

Discussion.—Diplostomulum desmognathi occurs free in the body cavity. In this respect it agrees with *D. ambystomae* Rankin and Hughes, 1936. These two species and *Diplostomulum trituri* Kelley, 1934, from the eye and brain cavities of *Triturus v. viridescens* (Raf.), are the only parasites of this genus that have been reported from amphibia as far as can be determined.

D. desmognathi is considerably smaller and more ovate than these other two species. The crura are much narrower than those of *D. ambystomae* and reach posterior to the holdfast organ. The hindbody rarely reaches the tubular proportions observed in *D. ambystomae*. The absence of lateral suckers distinguishes this species from most other known diplostomula.

The excretory system, as in other species of the genus, comprises two separate units, the flame-cell system dorsal to a reserve-bladder system. The urinary bladder fills most of the space between the posterior edge of the holdfast organ and end of the body. It is distinctly bilobed. The bladder empties to the outside through a dorso-

terminal pore. The halves of the excretory bladder extend anterolaterad to the holdfast organ as prominent tubules. The tubules receive the common trunks of the primary excretory apparatus at the anterior level of the holdfast organ. The primary excretory system is arranged according to the same plan as that in *D. ambystomae,* eighty-eight flame-cells, forty-four on each side.

The arrangement of tubules of the reserve-bladder system agrees superficially with that of *D. ambystomae.* There are four pairs of transverse commissural vessels, however, two more than in *D. ambystomae,* and an extra pair of lateral collecting vessels. All commissural vessels have dendritic branches. This condition does not occur in *D. gigas* Hughes and Berkhout, 1929, or in *D. ambystomae.* The only vessels not having these branches are the tubular portions of the bladder and short interconnecting tubules. The calcareous concretions are very numerous. An unusual feature is the apparent division of concretions into as many as four units in a single vesicle. Undeveloped vesicles, similar to those in *D. ambystomae,* were also observed.

NEMATODA

Superfamily: Spiruroidea Railliet and Henry, 1915
Family: Cucullanidae Cobbold, 1864
Sub-Family: Seuratinae Hall, 1916

Omeia Hsu, 1933

The genus Omeia was proposed by Hsu (1933) to contain the only species known, *Omeia hoepplii* Hsu, found in *Rana tibetana* from China. He placed it in the family Thelaziidae Railliet, 1916. Chitwood and Wehr (1934), in a study of the Spiruroidea, transferred it to the family Cucullanidae Cobbold, 1864. The generic characters must be emended to place this new species in the genus.

Genus Omeia Hsu, 1933, char. emend.

Generic diagnosis: *Cucullanidae.* Mouth with six dentigerously ridged small lips, followed by a buccal capsule which is triangular in cross-section; two amphids, four sub-medial papillae with double nerve endings, and six labial papillae; esophagus long and muscular throughout; intestinal caecum present. Male: tail pointed, without caudal alae; a large number of pre-anal papillae and four pairs of post-anal papillae; spicules similar and subequal; gubernaculum present. Female: oviparous; tail pointed, with or without coronet of papillae; vulva from middle of body posteriorwards; two uterine tubes; eggs with a thick smooth shell and more or less unsegmented contents at deposition. Parasites of the intestinal tract of frogs and salamanders.

Type Species: *Omeia hoepplii* Hsu, 1933.

Omeia papillocauda sp. nov.

Fig. 7

Description.—With characters of genus. Male: body length 3.95 mm., width 0.12 mm.; intestinal caecum 0.03 mm.; esophagus 0.41 mm.; tail 0.09 mm., very short pointed, bent ventrally; spicules equal and small; gubernaculum very small and triangular; large anal and three large pre-anal papillae; variable number of small pre-anal papillae. Female: body length 7.12 to 13.33 mm., width 0.33 to 0.54 mm.; esophagus 0.61 mm.; intestinal caecum 0.07 mm.; vulva to posterior end 4.04 mm.; vulva muscular, with prominent lips, located posterior to middle of body; eggs oval, 42.9 by 29.7 microns; tail 0.17 mm., short, pointed, with a coronet of four rows of papillae.

Host: *Desmognathus fuscus fuscus* (Raf.), *D. phoca* (Matthes), *D. quadramaculatus* (Holbrook), and *Gyrinophilus porphyriticus danielsi* (Blatchley).

Habitat: Rectum and lower intestine of host.

Locality: Second stream below Lake Powhatan flowing into Bent Creek, Pisgah National Forest, Buncombe County, North Carolina.

Type Specimens: Cotypes of *Omeia papillocauda* have been deposited in the United States National Museum.

Discussion.—Only one species of the genus Omeia has been hitherto described, *O. hoepplii* Hsu, 1933. The present species agrees with *O. hoepplii* as follows: finely striated cuticle; six small lips with dentigerous ridges around a thick-walled buccal capsule; two amphids; four prominent sub-medial papillae with double nerve endings; six labial papillae; four pairs of post-anal papillae in the male; vagina leads cephalad and divides into two uterine branches which run cephalad and caudad respectively.

O. papillocauda differs from *O. hoepplii* in the following respects:

	O. papillocauda	*O. hoepplii*
1.	Size small, 3.95 to 13.33 mm. by 0.12 to 0.54 mm.	Size large, 23.3 to 58.2 mm. by 0.51 to 0.88 mm.
2.	Six lips more or less fused to three.	Six distinct lips.
3.	Sub-medial papillae near periphery.	Sub-medial papillae near center.
4.	Intestinal caecum very short.	Intestinal caecum long.
5.	Male:	
	a. Spicules and gubernaculum small.	Spicules and gubernaculum large.
	b. Large anal and three large pre-anal papillae present.	No large papillae present.
6.	Female:	
	a. Vulva posterior to middle of body.	Vulva near middle of body.
	b. Vulva muscular and with prominent lips.	Vulva without prominent lips.
	c. Tail with coronet of four rows of papillae.	Tail without coronet.

Superfamily: Oxyuroidea Railliet, 1916
Family: Oxyuridae Cobbold, 1864
Sub-Family: Oxyurinae Hall, 1916

Oxyuris (s. l.) *magnavulvaris* sp. nov.

Fig. 8

A large number of female oxyurids were obtained from the intestine of several species of salamanders collected in the mountains of western North Carolina. Since no males have been found it has been difficult to place this species in the correct genus and it is with some hesitancy that it is described. It is belived, however, that the specific characters are striking enough to warrant such description.

Description.—Measurements given are averages from eight specimens mounted in glycerine. Length 5.25 mm., width 0.50 mm.; length of esophagus 0.53 mm.; diameter of esophagus bulb 0.11 mm.; vulva-tail distance 2.87 mm.; anus-tail distance 0.45 mm.; anterior body with strong annulations; mouth with triangular orifice, surrounded by three inner lips and six outer lips, these latter each with a small papilla; ovary encircling esophagus; large vulval papilla, 0.36 by 0.23 mm.

Host: *Desmognathus fuscus fuscus* (Raf.), *D. ochrophaeus carolinensis* Dunn, *D. phoca* (Matthes), *D. quadramaculatus* (Holbrook), *Eurycea bislineata wilderae* Dunn, *E. gutto-lineata* (Holbrook), *Plethodon cinereus* (Green), *P. glutinosus* (Green), *P. yonahlossee* Dunn, and *Triturus viridescens viridescens* (Raf.).

Habitat: Rectum and lower intestine of host.

Locality: Second stream below Lake Powhatan, emptying into Bent Creek, and Lake Powhatan, Pisgah National Forest, Buncombe County, North Carolina.

Type Specimens: Cotypes of *Oxyuris magnavulvaris* have been deposited in the United States National Museum.

Discussion.—Walton (1929) described a new species, *Pharyngodon batrachiensis* from the intestine of tadpoles of *Rana pipiens* Schreber on the basis of females only. *O. magnavulvaris* resembles *P. batrachiensis* in the following details: body short and stout, anterior end with distinct annulations, mostly surrounded by three lips, and esophagus with a prominent posterior bulb separated by a distinct constriction. It differs from *P. batrachiensis* in the larger size, non-spiked tail, three lips supplemented with six outer lips provided with small papillae, prominent vulval papilla, ovary entwined about esophagus, eggs with developing larvae at deposition, and eggs with a capsule at one end and a characteristic "plug" at the other.

The characters of the genus Pharyngodon, however, indicate that the present species does not belong there. The posterior end of the body is not obliquely truncate and provided with a long subulate tail; eggs not unsegmented; and the mouth has an outer circle of six lips. The only other genus that the new species might enter is Tachygonetria Wedl, 1862, for the mouth has six small lips, cuticle with annulations, and eggs with developing larvae at deposition, all characters of Tachygonetria. It differs from Tachygonetria, however, in practically all other respects.

It seems proper to place this species in the genus Oxyuris *sensu latu* until a male is found.

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Explanation of Plates

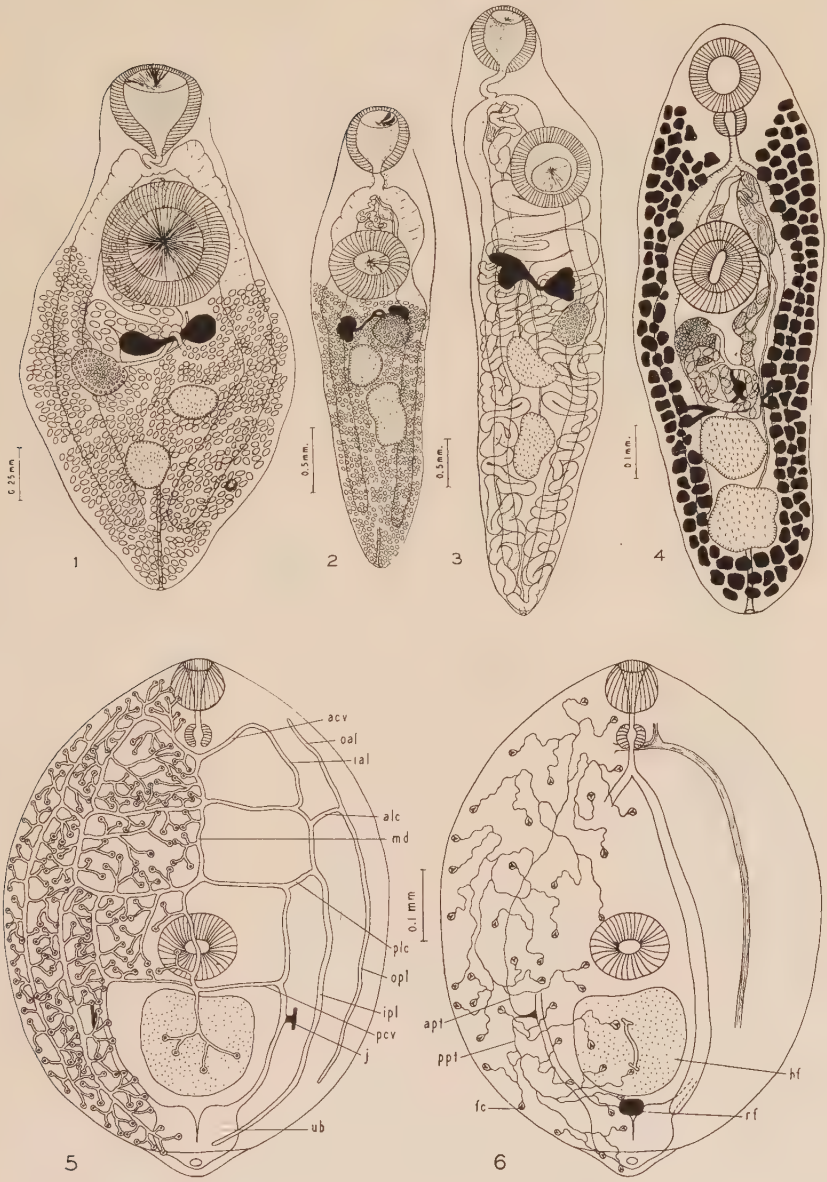
Plate I

Fig 1. *Phyllodistomum solidum* n. sp., ventral view.
Fig. 2. *Gorgoderina tenua* n. sp., ventral view.
Fig. 3. *Gorgoderina bilobata* n. sp., ventral view.
Fig. 4. *Allocreadium pseudotritoni* n. sp., ventral view.
Fig. 5. Semidiagrammatic representation, dorsal view of the reserve bladder system of *Diplostomulum desmognathi* n. sp. as seen in living specimens much flattened by cover-glass pressure.
Fig. 6. *Diplostomulum desmognathi* n. sp., ventral view, showing flame-cell pattern. Drawn from living material much flattened by cover-glass pressure, and supplemented with preserved specimens for further body details.

Plate II

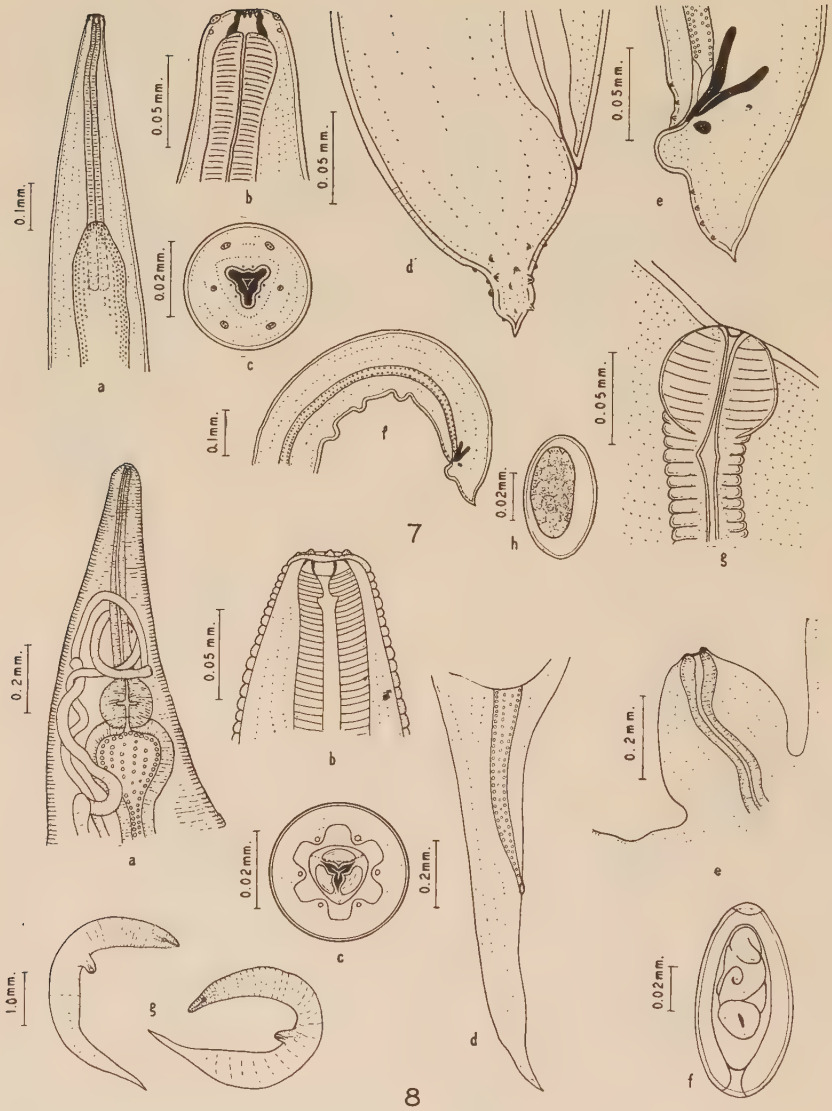
Fig. 7. *Omeia papillocauda* n. sp.
a. Anterior end of adult female.
b. Anterior end enlarged.
c. *en face* view.
d. Posterior end of female showing papillae.
e. Posterior end of male showing spicules and gubernaculum.
f. Posterior end of male showing three large pre-anal papillae.
g. Vulva of female.
h. Egg.
Fig. 8. *Oxyuris magnavulvaris* n. sp.
a. Anterior end of adult female.
b. Anterior end enlarged.
c. *en face* view.
d. Tail of female.
e. Vulva of female.
f. Egg.
g. Whole worms, showing proportionately large size of vulva.

PLATE I



Abbreviations used: acv, anterior transverse commissural excretory vessel, *alc,* anterior lateral collecting excretory vessel; *apt,* anterior primary collecting excretory tubule; *fc,* flame-cell; *hf,* holdfast organ; *inl,* inner anterior lateral excretory vessel; *ipl,* inner posterior excretory vessel; *j,* juncture of primary excretory apparatus with reserve bladder; *md,* median dorsal excretory vessel; *oal,* outer anterior lateral excretory vessel; *opl,* outer posterior lateral excretory vessel; *pc,* posterior transverse commissural excretory vessel; *plc,* posterior lateral collecting excretory vessel; *ppt,* posterior primary collecting excretory tubule; *rf,* fundament of reproductive organs; *ub,* urinary bladder.

PLATE II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THE PRODUCTION OF ARTIFICIAL IMMUNITY AGAINST HYDATID DISEASE IN SHEEP*

EDWARD L. TURNER, E. W. DENNIS AND D. A. BERBERIAN

There now seems to be little room for doubt that protective reactions of a degree can be produced against certain cestode parasites by means of artificial immunization. Miller (1930, 1931, 1932, 1934) and his collaborators Massie (1932), Gardiner (1932) and Kerr (1932) demonstrated the production of immunity against *Cysticercus fasciolaris* in rats. These investigators were able to demonstrate the production of immunity by means of active immunization with prepared antigen, by attempts to super-infest and by the use of specific immune sera. Evidence for the transmission of immunity from parent to offspring was also observed in some of their experiments. Kerr (1935) has recently published evidence of the production of immunity against another cestode parasite, *Cysticerus pisiformis,* in the rabbit. Kerr and Petkovich (1935) have produced immunity in rabbits against the liver fluke *Fasciola hepatica.*

Four years ago the authors began a study of the possibilities of the production of artificial immunity against *Echinococcus granulosus.* Attempts were made to study the production of immunity in the definitive and intermediate hosts. Data has been reported (Turner, Berberian and Dennis, 1933, 1935, 1936) in evidence of the fact that the definitive host (dog) shows a marked decrease in incidence and degree of intestinal infection with *Echinococcus granulosus* following the use of antigens prepared from hydatid cyst material. Partial, rather than complete protective reactions were observed in the definitive host.

Dévé (1927) was unable to produce experimental artificial immunity against *Echinococcus granulosus* in rabbits. In his experiments rabbits were immunized by the injection of sheep hydatid fluid and "hydatid sand." Following the injections of antigens living "hydatid sand" was introduced subcutaneously and three months later growing cysts were found on microscopic observation. Dévé (1933) has also reported failure in the experimental production of immunity in white rats by means of specific serotherapy and has been unable (Dévé, 1934) to produce any curative or preventative reaction in white rats or rabbits by means of sunbcutaneous injections of hydatid membrane material.

The present paper deals with the experimental immunization of sheep against the larval stage of the life cycle of *Echinococcus granulosus.*

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EXPERIMENTAL DATA

Material and Methods

A. *Animals*

In order to insure working with clean animals a group of pregnant ewes was purchased in the winter of 1932. These animals were placed on the Near East Foundation Farm at Talabaya where they were housed in specially constructed quarters.* Seventy ewes were under the care of a shepherd who was responsible solely to the authors. As the young lambs were born they were cared for separately by a second attendant, but were allowed to feed from their own mothers. Each lamb was marked with metal ear tags and with an adjustable leather collar on which a duplicate number was placed. The collars were secured with lead seals. Within one to three weeks after birth initial injections of antigen were given.

B. *Preparation and Dosage of Antigen*

The antigen was prepared by obtaining viable scolices and germinating membrane from fertile cysts of the lungs and livers of both sheep and cattle collected from the city abattoir. The scolices and germinating membrane were placed in an incubator at 37° C. and when thoroughly dry reduced to a fine powder. This powder was stored in tightly stoppered bottles. Immediately prior to use a 1 per cent suspension in 0.5 per cent phenol saline was prepared. There was some tendency for the suspension to precipitate so that it was necessary to agitate it frequently in order to obtain relatively uniform dosages in the syringe.

All injections of antigen were given into the muscles of the thigh. In all cases the initial dosage of antigen consisted of 0.5 cc. Subsequently injections were given at approximately weekly intervals. In order to determine the influence of the quantity of antigen received certain animals were given only one injection; others were given two, three, four or five doses. When given, the second dose consisted of 1.0 cc., the third of 2.0 cc., the fourth and fifth of 2.5 cc.

C. *Determination of Degree of Immunity, Experimental Infection*

1. Material Used for Infection

Following the completion of the injections of antigen, intervals of from 21 to 170 days were allowed before attempts were made to infect the animals. Infectious material was obtained from two sources, (a) experimentally infected dogs which gave us known positive stools, and (b) selected material from street dogs delivered to the laboratory. Material used for feeding consisted of a suspension of feces containing

* We are especially indebted to the Near East Foundation for their cooperation during the experiment.

known numbers of onchospheres per cubic centimeter. By feeding material which was one week, two weeks or one month old, an attempt was made to determine whether fresh material was as infectious as that in which the onchospheres had been passed for some time. The animals were given from 500 to 1000 onchospheres at the time of their infective feeding. The first few animals were given their infected feeding in milk from nursing bottles. This was found to be totally unsatisfactory as some of the material was lost through drooling, which tended to contaminate the vicinity. Bottle feedings were therefore replaced by direct feeding through a funnel and stomach tube. In this way a measured amount of the onchosphere suspension could be placed in the funnel and washed directly into the stomach by a small amount of water. There was thus relatively little danger of contamination from the mouth of the animal, and we do not believe any of the material was regurgitated.

2. Controls

The efficiency of immunization and the viability of the onchospheres was controlled by giving appropriate amounts of each lot of feeding material to non-immunized lambs.

3. Determination of Infection

Periods of 331 to 374 days were allowed to elapse between the feeding of infected material and the autopsy. As soon as the animals were killed, the viscera were completely removed. Careful gross examinations were made of the surface of the lungs, heart, liver, kidneys and spleen. Anterior and posterior aspects of the lungs, and the dorsal and ventral surfaces of each liver were photographed. Surface counts of cysts in each organ were then made. Each organ was then subjected to sectioning in strips from two to three millimeters in width so that any internally located cysts could also be counted. As is usually the case, the large majority of the cysts were located superficially. Representative and questionable cysts were removed from the organs of each animal in which infection was present, and fixed in Zenker's solution for subsequent histological study.

All animals in these groups were subjected to intradermal Casoni tests some time before autopsy. 0.5 cc. of hydatid fluid was injected intradermally on the wool-free region of the inner aspect of the thigh. Immediate reactions were read after 15 to 30 minutes, and delayed reactions were checked after 24 hours.

D. *Experimental Protocols*

A total of 115 animals have been studied in these experiments. These animals were divided into groups of from three to seven in number.

Each group consisted of one or more controls and lambs which had received a varied amount of antigen; all animals in each group received the same feeding material and were fed on the same day. The majority of the experiments were nullified and had to be discarded because of failure of feeding material. However, in other groups successful infection of the controls gave validity to the results. The results of the observations on the three groups where all factors were controlled and where there were no complications are presented in the following protocols.

GROUP I

Animal no. 15 (female) IMMUNIZED.
Antigen injections (1% antigen suspension intramuscularly).
March 21, 1933, 0.5 cc.
March 28, 1933, 1.0 cc.
April 4, 1933, 2.0 cc.
April 11, 1933, 2.5 cc.
Feeding on May 2, 1933, consisted of 500 onchospheres in suspension (material two weeks old). Feeding was 31 days after the last injection of antigen.
Casoni, March 3, 1934, immediate 2 plus, delayed 1 plus.
Autopsy, May 27, 1934 (390 days following feeding).
Liver, 25 cysts, all but one 2–3 millimeters diameter, one liver cyst 12 millimeters in diameter. All of these cysts were hard and the walls exceedingly well calcified.
Lungs, kidneys and spleen, negative.

Animal no. 22 (female) IMMUNIZED.
Antigen injections (1% antigen suspension intramuscularly).
March 21, 1933, 0.5 cc.
March 28, 1933, 1.0 cc.
April 4, 1933, 2.0 cc.
Feeding, May 2, 1933, consisted of 500 onchospheres of material identical in character with that given to animal no. 15. (28 days following the last antigen injection.)
Casoni not performed.
Autopsy, March 1, 1934 (303 days following feeding). This animal died following an attack of "staggers" and on autopsy was found to have *Coenurus cerebellaris* as the cause of death.
Liver, 10 cysts, 1–3 mm. in diameter, well calcified.
Lungs, 6 cysts, 1–4 mm. in diameter, well calcified.
Kidneys, spleen, brain, negative for hydatid cysts.

Animal no. 62 (male) CONTROL.
Feeding, May 2, 1933, consisted of material identical with that given to animals no. 15 and 22.
Casoni, March 3, 1934, immediate 2 plus, delayed 1 plus.
Autopsy, March 8, 1934 (331 days following feeding).
Liver, 68 cysts, 1–8 mm. diameter, no calcification.
Lungs, 48 cysts, 3–8 mm. diameter, no calcification.
Kidney, 1 cyst, 4×12 mm. diameter.
Spleen, 1 cyst, 6×10 mm. diameter.

GROUP II

Animal no. 3 (male) IMMUNIZED.
Antigen injections (1% antigen suspension intramuscularly).
February 18, 1933, 0.5 cc.

February 28, 1933, 1.0 cc.
March 7, 1933, 2.0 cc.
March 14, 1933, 2.5 cc.
March 21, 1933, 2.5 cc.

Feeding, May 23, 1933, consisted of 1000 onchospheres in suspension (mixture of material 1 month and 1 week old). 67 days following the last injection of antigen.

Casoni, March 3, 1934, immediate 4 plus, delayed 3 plus.

Autopsy, May 30, 1934 (372 days after feeding).
Liver, 190 cysts, 2–4 mm. diameter, mostly calcified.
Lungs, 50 cysts, 4–6 mm. diameter, mostly calcified.
Kidneys, 1 cyst, 3 mm. diameter, calcified.
Spleen, 2 cysts, 2 and 3 mm. diameter, calcified.

Animal no. 11 (female) IMMUNIZED.

Antigen injections (1% antigen suspension intramuscularly).
February 18, 1933, 0.5 cc.
February 28, 1933, 1.0 cc.
March 7, 1933, 2.0 cc.
March 14, 1933, 2.5 cc.

Feeding, May 23, 1933, consisted of 1000 onchospheres in suspension (mixture of material 1 month and 1 week old). 60 days following last injection of antigen.

Casoni, March 3, 1934, immediate 1 plus, delayed negative.

Autopsy, May 30, 1934 (372 days following feeding).
Liver, 137 cysts, 1–3 mm. diameter, 80% heavily calcified.
Lungs, 68 cysts, 2–3 mm. diameter, calcified.
Kidneys, 2 cysts.
Spleen, 1 cyst.

Animal no. 23 (female) IMMUNIZED.

Antigen injections (1% antigen suspension intramuscularly).
March 14, 1933, 0.5 cc.
March 21, 1933, 1.0 cc.
March 28, 1933, 2.0 cc.

Feeding, May 23, 1933, consisted of 1000 onchospheres in suspension (mixture of material 1 week and 1 month old), 56 days following last injection of antigen.

Casoni, March 3, 1934, immediate 3 plus, delayed 1 plus.

Autopsy, May 31, 1934, (373 days following feeding).
Liver, 18 cysts 3–5 mm. diameter, 1 cyst 10 mm. in diameter (all heavily calcified).
Lungs, 26 cysts about the same diameter and condition.
Kidneys and spleen negative.

Animal no. 32 (female) IMMUNIZED.

Antigen injection (1% antigen suspension intramuscularly).
March 21, 1933, 0.5 cc.
March 28, 1933, 1.0 cc.

Feeding, May 23, 1933, consisted of 1000 onchospheres in suspension (mixture of material 1 week and 1 month old). 56 days following last antigen injection.

Casoni, March 3, 1934, immediate 2 plus, delayed 2 plus.

Autopsy, May 31, 1934 (373 days following feeding).
Liver, 25 cysts, 2–5 mm. diameter, highly calcified.
Lungs, 66 cysts, 2–6 mm. diameter, highly calcified.
Kidneys, negative.
Spleen, 1 cyst 3 mm., highly calcified.

Animal no. 42 (male) IMMUNIZED.
Antigen injection (1% antigen suspension intramuscularly).
March 21, 1933, 0.5 cc.
Feeding, May 23, 1933, consisted of 1000 onchospheres in suspension (mixture of material 1 week and 1 month old), 67 days following the injection of antigen.
Casoni, March 3, 1934, immediate 3 plus, delayed 3 plus.
Autopsy, June 1, 1934 (374 days following feeding).
Liver, 49 cysts, 1–4 mm. diameter, highly calcified.
Lungs, 17 cysts, 2–5 mm. diameter, highly calcified.
Kidneys and spleen, negative.

Animal no. 51 (female) IMMUNIZED.
Antigen injections (1% antigen suspension intramuscularly).
March 21, 1933, 0.5 cc.
April 4, 1933, 1.0 cc.
April 11, 1933, 2.0 cc.
April 18, 1933, 2.5 cc.
May 2, 1933, 2.5 cc.
Feeding, May 23, 1933, consisted of 1000 onchospheres in suspension (mixture of material 1 week and 1 month old), 21 days following last injection of antigen.
Casoni, March 3, 1934, immediate 3 plus, delayed negative.
Autopsy, June 1, 1934 (374 days following feeding).
Liver, 45 cysts, 2–4 mm. diameter, 80% highly calcified.
Lungs, 51 cysts, 2–5 mm. diameter, highly calcified.
Kidneys and spleen, negative.

Animal no. 63 (male) CONTROL.
Feeding, May 23, 1933, consisted of material identical in source and character to that fed to animals nos. 3, 11, 23, 32, 42 and 51.
Casoni, March 3, 1934, immediate 3 plus, delayed 1 plus.
Autopsy, May 29, 1934 (371 days following feeding).
Liver, 252 cysts, 2–7 mm. diameter.
Lungs, 96 cysts, 3–8 mm. diameter.
Kidneys, 1 cyst in each kidney approximately 4 mm. diameter.
Spleen, negative
None of the cysts showed evidence of calcification and they all appeared to be normally growing hydatid cysts.

Group III

Animal no. 14 (male) IMMUNIZED.
Antigen injections (1% antigen suspension intramuscularly).
February 18, 1933, 0.5 cc.
February 28, 1933, 2.0 cc.
March 7, 1933, 1.0 cc.
March 14, 1933, 2.5 cc.
Feeding, August 31, 1933, consisted of 500 onchospheres in suspension (freshly collected material), 170 days following last injection of antigen.
Casoni, March 3, 1934, immediate 3 plus, delayed negative.
Autopsy, August 7, 1934 (341 days following feeding).
Liver, 53 cysts, 2–4 mm. diameter, all highly calcified.
Lungs, 13 cysts, 2–4 mm. diameter.
Kidneys and spleen, negative.

Animal no. 26 (male) IMMUNIZED.
Antigen injections (1% antigen suspension intramuscularly).
March 14, 1933, 0.5 cc.

March 21, 1933, 1.0 cc.
March 28, 1933, 2.0 cc.
Feeding, August 31, 1933, consisted of 500 onchospheres in suspension (freshly collected material), 157 days following last injection of antigen.
Casoni, March 3, 1934, immediate 3 plus, delayed 2 plus.
Autopsy, August 7, 1934 (341 days following feeding).
Liver, 49 cysts, 2–4 mm. diameter, well calcified.
Lungs, 12 cysts, calcified and obviously degenerating.
Kidneys and spleen, negative.

Animal no. 34 (male) IMMUNIZED.
Antigen injections (1% antigen suspension intramuscularly).
March 21, 1933, 0.5 cc.
March 28, 1933, 1.0 cc.
Feeding, August 31, 1933, consisted of 500 onchospheres in suspension (freshly collected material), 156 days following last injection of antigen.
Casoni, March 3, 1934, immediate 2 plus, delayed 2 plus.
Autopsy, August 13, 1934 (337 days following feeding).
Liver, 10 cysts, 2–3 mm. diameter, highly calcified.
Lungs, 2 cysts.
Kidneys and spleen, negative.

Animal no. 67 (female) CONTROL.
Feeding, August 31, 1933, consisted of material identical in source and character to that fed to animals nos. 14, 26, and 34.
Casoni, March 3, 1933, immediate 3 plus, delayed negative.
Autopsy, August 1, 1934 (335 days following feeding).
Liver, 32 cysts, 2–8 mm. diameter, evidence of some calcification.
Lungs, 22 cysts.
Kidneys and spleen, negative.

E. *Results*

In Group I the control animal had a total of 118 cysts in the liver, lungs, kidneys and spleen. Animal no. 15 which received four injections of antigen, totalling 6.0 cc. of the 1 per cent antigen suspension, had only 25 cysts in the liver and none elsewhere. Animal no. 22 which received three injections of antigen (3.5 cc.) had a total count of 16 cysts. The maximum cyst diameter seen in the control animal was from 8–12 mm. The largest cysts observed in the two immunized animals of this group were not over 4 mm. in diameter. The cysts in the control animal were not calcified while those seen in the two immunized sheep were hard and, when cut open with a knife, were obviously calcified. Immunized sheep no. 15 and control sheep no. 62 developed similar Casoni reactions.

Animals in Group II received twice as many onchospheres in their infected feedings as were given to those in Group I. The total number of cysts developing in these two groups showed a close correlation between the size of the infected feeding and the subsequent infection. Control no. 62 of Group I received 500 onchospheres in its feeding and developed 118 cysts while control no. 63 of Group II received 1000 onchospheres and developed a total of 349 cysts. The average number of cysts developing in the immunized animals of Group I was 20 while in

Group II the average number of cysts found in the immunized sheep was 125. All of the animals that had received injections of antigen showed marked evidence of cyst calcification while the cysts of the controls of Group II were thinner walled and lacked gross evidence of calcification. The largest cysts in Group II were found in the control no. 63 where a maximum diameter of 8 mm. was observed in lung cysts. The maximum diameter of the cysts in the immunized animals in this group varied between 4 and 6 mm. All of the immediate Casoni reactions in Group II were positive. However, immunized animal no. 11 with a total of 208 cysts gave only 1 plus immediate reaction and a negative delayed reaction.

In Group III the control animal no. 67 developed only 54 cysts while two of the immunized animals (no. 14 and no. 26) developed 66 and 61 cysts. The maximum diameter of the cysts observed in the control was 8 mm. while the immunized animals did not develop cysts greater in width than 4 mm. Calcification was much more marked in the cyst walls of the immunized animals. All immediate Casoni reactions in this Group were positive.

One consistent observation in these protocols is the fact that sheep receiving one, two or three injections of antigen developed relatively fewer cysts than those receiving four or five injections.

Microscopic studies of the cysts revealed marked differences in the structures of the cysts of the control and immunized animals. Control animals in all instances developed cysts that showed normal walls and an active germinal layer while in most instances the cyst structures in the immunized animals showed marked degenerative changes (see discussion and plates).

In addition to the detailed protocols presented above, the results of one entire series of experiments (Series 1935–1936) are given in condensed tabular form (Table I) to illustrate the types of variation in results encountered during the course of our study. The complement fixation test, Casoni test, and precipitin test were used to obtain a certain amount of data pertaining to the specific immunological responses. However, it was not until this series was nearly finished that we were able to prepare an antigen which we considered satisfactory for a quantitative study, consequently the data on this series is qualitative and designed to show immunological differences toward the termination of the experiment. The antigen used for obtaining the data given in Table I was a solution of hydatid protein obtained by treatment of hydatid cyst fluid with acetic acid. The preparation and properties of this purified antigen will be described separately by one of us (Dennis, 1937).

In the 1935–1936 series, 26 lambs which were maintained under the same conditions as those reported in the protocols above were immunized.

Each lamb received a total of 11–15 cc. of scolex suspension administered in 3 doses given intramuscularly. These animals were experimentally infected, by the stomach tube method, from 35 to 69 days after the last injection of antigen (for economy of space the time between the injections and the feedings are not shown since there appeared to be no relationship between these periods and the results). Nonimmunized lambs were given comparable feedings of echinococcus ova as controls. Variation in the age and number of ova administered is indicated in the table.

The results of this experiment are shown in the table. In the immunized sheep (nos. 301–310 inclusive) the average number of cysts per animal was 19.2 as compared with 5.25 for each of the control group. In this case it would appear that the injections of antigen actually increased susceptibility until one compares the condition of the cysts. In the immunized animals the cysts were uniformly small, calcified and degenerate; in the control group the cysts, although few, were normal when examined microscopically. In the immunized sheep (nos. 311–328 inclusive) there were but 2.6 cysts per animal as compared with an average of 5 cysts per animal in the control group. Again the microscopic examination of the cysts from these animals showed calcification and degeneration of the cysts in the immunized sheep, whereas the cysts in the control group were entirely normal. On analysis of the data, it would appear that a greater viability of the 12-day-old ova included within the feeding material of sheep 301–310 might explain the increased number of cysts per animal, although we hesitate to interpret data showing such a high degree of variability. The most significant and always constant finding is the degenerate nature of the cysts in the immunized animals as compared with the controls.

DISCUSSION AND CONCLUSIONS

Several very clear cut evidences of a protective reaction resulting from immunization are to be noted on analysis of the foregoing protocols. In general the total number of cysts counted in the immunized animals is far below that in the controls. This is clearly illustrated in the animals of Group I. The total count in the control is 118 cysts while animals no. 15 and no. 22 (immunized) have total counts of 25 and 16 cysts respectively. A similar difference is noted in Group II. In Group III the total number of cysts in the control is less than the count in some of the immunized animals but this feature is counterbalanced by the marked difference in the condition of the cyst structures on gross and microscopic examination.

Invariably the diameter of the cysts in the control animals is greater than that of the cysts in sheep that have received injections of antigen.

This difference in diameter varies from 50 to 100 per cent. Dew (1928) states that at five months the hydatid follicle may be about 1.0 cm. in diameter. The growth rate of cysts observed in our studies is obviously much slower as we rarely observed cysts 1.0 cm. in diameter at eleven or twelve months after infection. This must be interpreted as representing a definitely slower growth rate in our series than that seen by the Australian observer.

Cysts in immunized animals are firmer and more calcified than those in the controls. This difference is not only apparent on gross examination but is also obvious on cutting through the organs containing the cysts.

Another constant observation on gross examination is the difference in the numbers of deeply seated and superficial cysts in the control and immunized animals. Deep liver cysts are abundant in the control animals while most of the cysts counted in the immunized animals are superficially located. This tendency is probably the result of less resistance in the liver surface as compared to the interior of the organ. Hexacanth embryos lodging deeply in the liver tissue of immunized animals encounter a more active resistance than those lodging peripherally. The more superficially placed ones encounter resistance from only the deeper surface when growth is once started.

Although the gross differences are marked, and in most cases convincing, the microscopic differences observed on studying the cyst structures in the control and immunized animals are far more conclusive. In the control animals the cyst wall structures are clearly differentiated and normal in appearance. A normal active germinal layer is present. Cysts from immunized animals have much thicker walls. The laminated membrane and adventitial structures are not clearly differentiated and in many instances appear to be an undifferentiated "hyaline-like" mass. The germinal layer is absent or shows marked evidences of degeneration and there is a tendency towards obliteration of the lumen of the cysts. The difference in calcification of the cysts is also clearly defined microscopically. Scattered patches of calcification are observed in a few of the control cysts. This calcification is at least twice as marked in the most poorly calcified cysts of immunized animals. The process of calcification apparently starts in small islands around and in the cyst wall structures (adventitia). These islands of calcification then spread circularly around the cyst wall and tend to coalesce as the cyst grows older. In Group III where the control animal (no. 67) has a lower cyst count than the immunized animals (no. 14 and no. 26) these microscopic differences are fully as marked as they are in the other groups. In fact, these microscopic differences are constant throughout the entire series of experi-

ments as well as in the three groups reported in the protocols of this paper (see Plates).

As indicated in the results, one of the most interesting features in studying the protocols is the relationship of the number of injections of antigen to the number of cysts developed. Consistently, animals receiving only one, two or three injections of antigen developed fewer cysts than those receiving four and five injections.

Despite the fact that the accumulated data is inadequate for the formulation of any sweeping conclusions concerning the mechanism of resistance against *Echinococcus granulosus,* certain aspects of the problem are worthy of presentation.

Certain nonspecific factors probably play a significant rôle in determining whether or not infection occurs. Experimental evidence is accumulating in our laboratory indicating that the hatching of the onchosphere from the egg is greatly influenced by the hydrogen ion concentration and by the quality of the digestive juices to which they are exposed. It has been shown that qualitative differences exist between the intestinal juices of naturally suitable and unsuitable hosts which may reasonably account for differences in susceptibility of certain species of hosts under natural conditions. Furthermore individual differences in amount of free acid in the stomach and in the rate of passage of food through the alimentary tract at the time ova are ingested are probably important factors influencing the establishment of the infection. An additional nonspecific factor which appears to have been responsible for our failure to establish infection in certain experiments is that of the age and viability of the ova. Material which was fresh (24–48 hours old), and material kept in aqueous suspension at room temperature for one month or longer were unsatisfactory for establishing infection; material which was between 7 and 14 days old was usually more effective. The factors controlling the viability of the ova of echinococcus under natural and experimental conditions require further study.

Specific humoral antibodies and/or the increased tissue response which is the result of specific sensitization are alternative or correlative factors which may be responsible for the increased resistance to *Echinococcus granulosus* which results from the parenteral introduction of echinococcus antigen. It is well known (Fleig and Lisbonne, 1907; Welsh, Chapman and Storey, 1908; Fairley, 1923) that specific precipitins occur in the sera of individuals suffering from hydatid cysts. Also Ghedini (1906), Weinberg (1909) and Fairley (1922) amply demonstrated the presence of complement fixing antibodies in the sera of hosts suffering from hydatid disease. Clinical experience indicates that helminthicidal antibodies either are not produced or are unable to penetrate a normal hydatid cyst. Casoni (1911) was able to demonstrate specific

TABLE I.—*Results of Series 1935–1936*

No.	Age of ova	No. of ova	Casoni test	Unit of complement fixed	Ppt. test	Cysts in		Condition of cysts
						Liver	Lungs	
301	1 : 4 ; 12	3/1300	+++	8	++	12	8	calcified : 2–4 mm.
302	"	3/1300	++	8	+++	58	27	" 1–4 mm.
303	"	3/1300	++	6	+++	8	5	" 3–4 mm.
304	"	3/1300	+	6	+	23	7	" 2–4 mm.
305	"	3/2300	+++	6	++	23	0	" 2–4 mm.
306	"	3/2300	+	6	+++	8	0	" 2–4 mm.
307	"	3/3300	++	6	+++	0	5	" 2–4 mm. ; 1 was 7
308	"	3/3300	++	ac	+	4	0	" 3–4 mm.
309	"	3/3300	+	2	+++	4	0	" 2–3 mm.
310	"	3/3300	+++	8	+++	0	0	
330*c*	"	3/1300	0	ac	++	2	3	not calcif. ; 5–20 mm.
331*c*	"	3/2300	+	6	+++	0	0	
332*c*	"	3/3300	+++	8	+++	11	2	normal 3–6 mm.
333*c*	15	1/1500	+	2	++	0	3	" 3–4 mm.
311	1 : 3	2/1800	+	8	+++	4	0	calcified ; 3–5 mm.
312	"	2/1800	++	8	+++	6	1	" 4–5 mm.
314	1	2/3800	+++	8	+++	3	1	" 2–5 mm.
315	1	3/3800	±	8	++++	2	3	" 4–5 mm.
316	1	2/2800	++	8	+++	1	0	" 10 mm.
317	1	2/2800	+++	3	+++	0	2	" 3 mm.
318	1	2/2800	±	6	++	5	3	" 3–6 mm.
319	1	1/2000	+	8	±	2	2	" 2 mm.
320	1	1/3000	+++	6	++	0	0	
321	1	1/3000	++	6	+	1	0	" 8 mm.
322	1	1/3000	+	3	+++	0	0	
324	1	1/10,000	++	6	++	0	0	
325	1	1/10,000	++	6	+++	1	0	" 4 mm.
326	1	1/10,000	±	6	+++	0	0	
327	1	1/10,000	+++	8	++++			
328	1	1/3000	+++	8	+++	00	0	
334*c*	1 : 3	2/1800	+	6	+++	2		partial calcif ; 5–6 mm.
335*c*	1 : 3	2/1800	++	6	+++	3	5	normal ; 6–10 mm.
336*c*	1	1/3000	0	ac	+++	1	0	" 4 mm.
337*c*	1	1/3000	+	0	+++	6	2	" 4–6 mm.
338*c*	1	1/10,000	+++	8	++	1	5	" 4–8 mm.

In the first column the letter *c* indicates the control of sheep for the group above. In the second column the numerals indicate the age of the fecal suspension in days ; where more than one number is present the feeding material was a mixture. In the third column the numerator indicates the number of feedings one week apart, the denominator indicates the *total* number of echinococcus ova fed. Negative Casoni reaction indicated by 0. The complement fixations tests were quantitative ; *ac* indicates that the serum was anticomplementary. The precipitin tests were qualitative, not quantitative, using purified antigen 1 : 1000. Animals sacrificed 11–12 months after infection.

tissue "hypersensitivity" in hydatid infection by the intradermal injection of small quantities of hydatid fluid as the eliciting antigen.

It is our opinion that such degree of immunity as may result from artificial immunization under the conditions of our experiment is the expression of induced specific tissue sensitivity to hydatid antigen. This opinion is supported by the following observations:

1. The immunizing procedure did not prevent infection.
2. Immunization reduced the number of cysts, as compared with controls, only in certain experiments.
3. There were no significant differences in antibody content of immunized and control sheep under the stimulus of infection (Table I).
4. In all experiments immunized animals have shown marked ability to defend themselves against the growth of the parasites by a

rapid and highly efficient walling off of the invaders. The greatly thickened adventitia and thorough calcification of the wall of the hydatid cyst at an early period produces an efficient and eventually impermeable mechanical barrier, leading to death of the parasite as the result of starvation and the accumulation of toxic metabolites.

5. Hypersensitivity in resistant animals could always be demonstrated by the intradermal Casoni test. Control animals which were successfully infected usually but not always gave a positive Casoni test. It would seem that the previous sensitization (immunization) of the experimental sheep gave them the advantage of a more intense early response which was responsible for the observed differences in susceptibility.

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Explanation of Plates

All illustrations are photomicrographs taken with a Leica. Sections stained with haematoxylin-eosin. All cysts shown are approximately 1 year old.

Plate I

Fig. 1. Section through two continuous hydatid cysts in the lung of control sheep no. 66. Note the relatively thin cyst wall and absence of calcification in a normal cyst. ×20.

Fig. 2. Enlargement of a portion of fig. 1 to show normal structure of cyst wall and active germinal layer. ×50.

Figs. 3 and 4. Hydatid cysts in lung of immunized sheep no. 32. Note the greatly thickened wall, early calcification, and tendency toward obliteration of the lumina of the cysts. ×30.

PLATE I

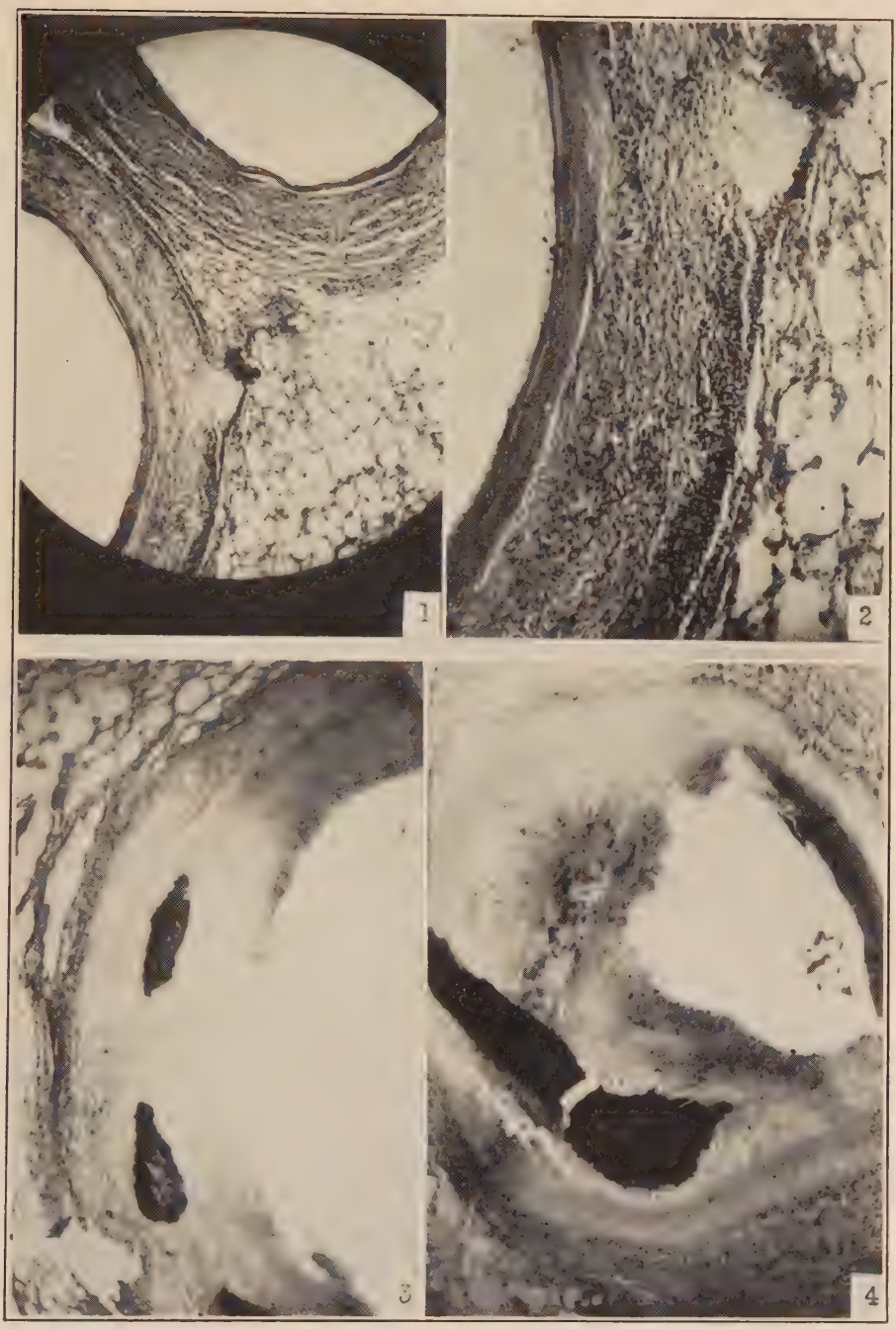


Plate II

Fig. 5. Cyst deep in liver of control sheep no. 63. Cyst has unusually thick wall and a small point of calcification in the adventitia. Germinative membrane normal and active. ×20.
Fig. 6. Normal thin walled cyst in the liver of control sheep no. 63 showing active germinative membrane. No calcifications. ×20.
Fig. 7. Germinative membrane and laminated layer of cyst in Fig. 5.
Fig. 8. Active germinative membrane of cyst shown in Figs. 5 and 7. ×200.

Plate III

Figs. 9, 10, 11 and 12. Illustrating the greatly thickened cyst walls, calcifications, and degeneration of germinative membrane in cysts of immunized sheep nos. 3, 23, 42 and 51 respectively

Plate IV

Fig. 13. Thick walled degenerative cyst in the liver of immunized sheep no. 42. ×10.
Fig. 14. Normal cyst in the liver of control sheep no. 63. ×10.
Fig. 15. Degenerating scolices and germinative membrane of cyst in liver of immunized sheep no. 3. ×500.
Fig. 16. Normal active proliferating germinal membrane in the cyst shown in Fig. 14. ×200.

PLATE II

PLATE III

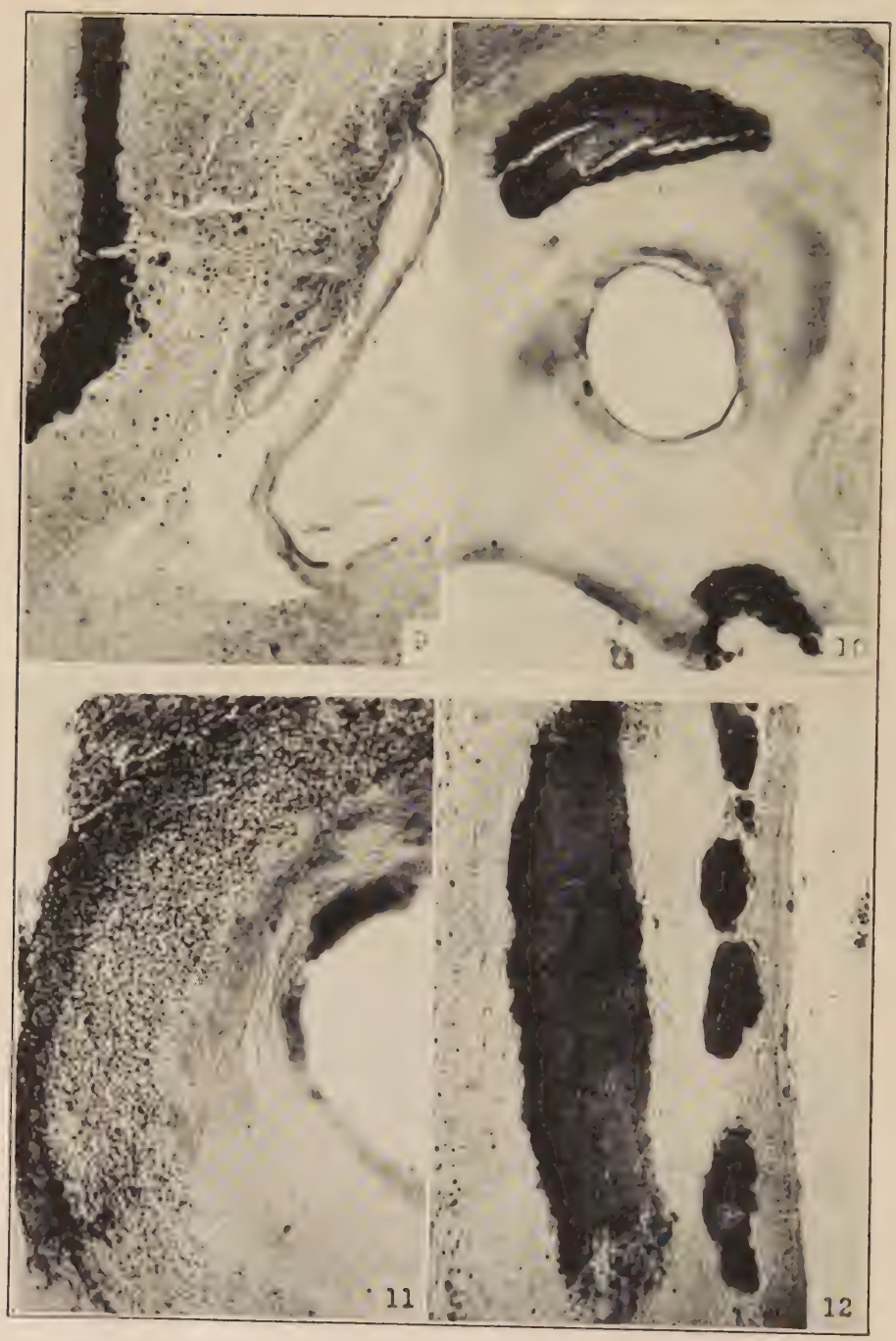
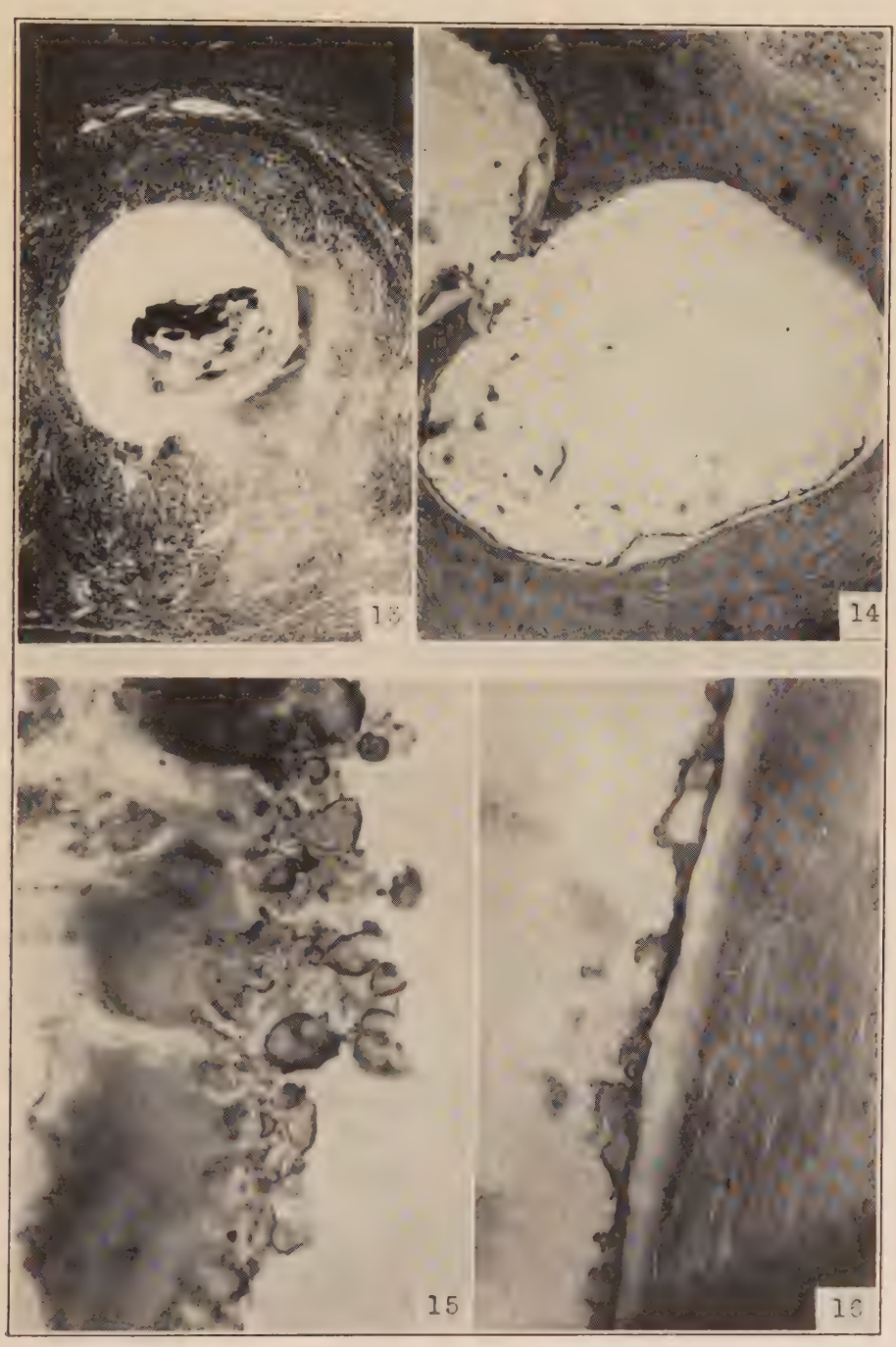


PLATE IV



A STABLE CONCENTRATED PURIFIED ANTIGEN FOR THE IMMUNOLOGICAL STUDY OF HYDATID DISEASE

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INTRODUCTION

Additional knowledge of the immunological phenomena which may occur in either the definitive or intermediate hosts as the result of their infestation with the adult or larval stages of *Echinococcus granulosus* may yield important information as to the nature of immunity resulting from infestation by helminths in general. Specifically, such knowledge may make possible specific immuno-prophylaxis in the case of either or both hosts, and thus facilitate the control of echinococcosis. Also, such knowledge may facilitate the clinical-laboratory diagnosis of hydatid disease of man. Very little is known about the nature of immunity to helminths and the data which is available is scanty and prone to be contradictory in nature. Recently Turner, Berberian and Dennis (1933, 1936) have shown that it is possible to artificially immunize dogs against *Echinococcus granulosus*. Encouraging results, although frequently irregular, have been obtained from experiments on the artificial immunization of sheep against echinococcosis (Turner, Dennis and Berberian, 1937). The utilization of immunological phenomena in the clinical-laboratory diagnosis of helminthiasis has been more extensively applied, especially in the case of hydatid disease of man.

The most essential factor to the extension of our knowledge of immunity to helminths is to have available an antigen which is specific, and of which the nature and serological properties are known. Furthermore, a desirable antigen should be sufficiently pure and stable in form to permit its use in quantitative as well as qualitative determinations of the immunological responses of a host. In addition to these qualifications, an antigen of such versatility that it could be used in all three of the methods available for the study of immunity to helminths, namely for the detection of precipitins, for the fixation of complement, and for the demonstration of a specific allergic state, would make it possible to compare and analyze these different manifestations of immunological response.

The substance that has been most commonly used as the antigen for the detection of precipitins and complement-fixing antibodies in the serum of patients and animals suffering from hydatid disease, and for the demonstration of specific allergy, is bacteriologically sterile fluid

which has been aspirated from fresh fertile hydatid cysts. Fleig and Lisbonne (1907), and Welsh, Chapman and Storey (1908) who first utilized the precipitin test in hydatid disease used hydatid fluid from human sources, and the latter authors pointed out that variation in the quality of hydatid fluids used as antigens in this test might account for a portion of the negative results. Fairley (1922) demonstrated that cysts from human sources are likely to yield fluid with little or no antigenic properties.

Fairley (1923) demonstrated that the antigen which was most reliable for use in the precipitin test was to be obtained by sterile puncture of fresh cysts of the viscera of sheep, and could be kept free of bacterial growth and antigenically stable for several months by the addition of phenol to a concentration of 0.5 per cent. Dew (1928) states that if K. D. Fairley's technic is used, but that definite precipitates alone be relied upon, the test will be positive in about 65 per cent of all cases of hydatid disease. Obviously a margin of error of 35 per cent is not permissible in a satisfactory test used for diagnostic purposes.

N. H. Fairley (1922) followed the lead of Weinberg (1909) and Ghedini (1906) in applying the principles of the complement-fixation reaction to the study of hydatid disease. Fairley standardized the technic, defined the scope and limitations of the complement fixation test, and placed it on a more acceptable quantitative basis. This author introduced the use of alcoholic extract of scolices as a highly sensitive antigen, but he adhered to the use of pure hydatid fluid, without preservative, as the antigen used in routine laboratory diagnoses. The test as developed by Fairley is highly sensitive but has certain disadvantages, namely the necessity of standardizing each lot of hydatid fluid antigen in the presence of hydatid, normal and syphilitic sera before use; the fact that many hydatid fluids favor the hemolytic action of complement; and the more sensitive type of antigen, the alcoholic scolex antigen, is tedious to make and prone to give pseudopositive readings, particularly with syphilitic sera.

It is apparent from this brief review of the literature that accurate quantitative serological studies of hydatid disease are hampered by the use of an antigen of such a variable nature as pure hydatid fluid. Furthermore, the use of such a variable antigen in a test of diagnostic significance is highly undesirable. Several years ago Dean and Webb (1926) pointed out the necessity of antigen and antibodies being used in *optimal* proportions, since an excess of either inhibits either precipitation or the fixation of complement. This consideration appears to have been neglected until Goldsworthy (1928) pointed out that the failure to have optimal proportions between hydatid fluid antigen and antiserum may be responsible for falsely negative results.

In connection with current experiments on the immunization of dogs and sheep against *Echinococcus granulosus* the antigen described below has been developed and has the qualities desirable for its use in quantitative studies; it is uniform; it is stable; and it is equally suitable for use in the precipitin test, for the fixation of complement, and for the allergic skin test of Casoni (1911).

MATERIAL AND METHODS

Preparation of the antigen. For the preparation of our antigen we have used freshly aspirated bacteriologically sterile hydatid fluid from cysts of the liver and lungs of both cattle and sheep as the source of material. This fluid in lots of approximately one liter is chilled in the ice-box and crystalline trichloracetic acid is added to a concentration of five per cent. Three per cent trichloracetic acid does not precipitate all of the antigenic material; eight per cent trichloracetic acid yields a larger precipitate, but with only a slight increase in potent material. The acidified hydatid fluid is placed in the ice-box (4° C.) for several hours, or over night. After flocculation has been completed in the cold, the precipitate is collected by centrifugalization in 250 cc. centrifuge bottles, finally concentrated in one bottle and washed three times in distilled water to remove excess acid. The precipitate is then suspended in about 50 cc. of distilled water, and 10 per cent NaOH solution is added drop by drop, with constant agitation, until solution has been completed. There is usually some insoluble residue which is collected by centrifugalization and discarded. The clear supernatant is then chilled and subjected to reprecipitation by the addition of 1/N glacial acetic acid until precipitation is complete. After standing in the ice-box over night, the precipitate is collected by centrifugalization, washed free of excess acid with distilled water, placed in an evaporating dish and dried in a drying oven at 37° C., or dried over calcium chloride. The dried precipitate is ground in a fine mortar and the resulting white or very light brown amorphous powder is stored in a $CaCl_2$ dessicator in the dark. The yield varies a great deal for different lots of hydatid fluid, but is usually in the neighborhood of 100 mgms. per liter of fluid.

Use of the antigen. For use, the purified hydatid protein is dissolved in sterile slightly alkaline physiological saline in the proportions of 1 mgm. per cc. (1 : 1000). If it is desirable to make up a stock solution to be used over a period of several days, the fluid may be sterilized by filtration through a Seitz EK filter, or bacteriostasis may be obtained by the addition of 0.5 per cent chloroform. This concentration of 1 : 1000 represents approximately a tenfold concentration of the antigenic material of hydatid fluid of sufficient potency for serological work.

The precipitin test with purified hydatid antigen. For the precipitin test we prefer to use small tubes 50 mm. in length, with an inside diameter of 5 mm. Fermentation vials are excellent for this purpose. If antigen or antiserum is small in amount, small tubes 3×35 mm. may be made from capillary tubing. The serum is cleared by centrifugalization at high speed for 20 minutes, and is introduced into the bottom of the tubes to a depth of 8–10 mm. by means of a capillary pipette equipped with a 1 ml. rubber nipple. The serum should be carefully introduced so that it is not smeared on the sides of the tube. The tubes are set up in plasticine instead of in a rack. The antigen solution is then carefully layered onto the surface of the serum. If the serum is very active, a precipitate appears at the interface of the serum and antigen almost immediately. Precipitation is usually complete by the end of two hours at room temperature. During cold weather we have found it advantageous to place the tubes in the incubator at 28–30° C. for 30 minutes, and then leave them at room temperature for an hour and a half. Readings after 24 hours (or over night in the ice-box) have yielded but little additional information.

For routine clinical-laboratory diagnosis the hydatid antigen should be used in three different concentrations, namely, 1 : 1000; 1 : 10,000; 1 : 50,000, using a constant volume of antiserum, to control the possibility of an excess of antigen inhibiting the reaction in the presence of a weak antiserum. The precipitin test is absolutely specific.

Quantitative studies are carried out by using a constant volume of antiserum and graduated falling concentrations of antigen. The dilution of antigen (*e.g.,* 1 : 10,000) at which maximum precipitation occurs is taken as the titer.

The complement-fixation test with purified hydatid antigen. Purified hydatid protein in neutral saline solution is a suitable antigen for the fixation of complement. The test may be carried out as described by Dew (1928), or by using the Kolmer modification of the Wassermann test (Kolmer and Boerner, 1933). In either case we have found that a 1 : 5000 solution of hydatid protein behaves as a specific antigen; it is sensitive, it is not anticomplementary and will not ordinarily react with syphilitic sera. A study of 50 syphilitic sera did not yield a single pseudo-positive reaction. However, controls of both normal and syphilitic sera should be used, and should the antigen react with the syphilitic serum the test should be repeated using a more dilute solution of the antigen (1 : 10,000).

THE CASONI TEST WITH PURIFIED HYDATID ANTIGEN

Sterile hydatid protein antigen solution 1 : 10,000 is an excellent antigen for the intradermal test for specific allergy. The test is made by

injecting 0.2 cc. of 1 : 10,000 antigen solution intradermally into the skin of the inner aspect of the forearm. The antigen elicits either the immediate wheal or the delayed inflammatory reaction, or both, when injected into a sensitized subject.

THE RELIABILITY OF THE ANTIGEN IN DIAGNOSIS

Purified hydatid protein antigen has thus far been used in the serological examination of ten hospitalized patients. The results of the precipitin, complement-fixation, and Casoni tests were uniformly strongly positive in nine of the ten cases. The tenth case was that of a six-year-old child with an hydatid cyst of the lung which had undergone suppuration, and as is usual in such cases (Dew, 1928) the immunological tests were all negative. The correctness of the results of the serological examinations of the nine patients who were positive were fully confirmed at operation.

Thirty-three sheep which were experimentally infected with hydatid disease have been studied by means of the precipitin, complement-fixation and Casoni tests as described above. Two of the sheep were negative by all three methods of immunological examination, and no cysts were found when the animals were sacrificed 13 months after the date of the infective feeding of onchospheres. The other 31 animals were positive by both the Casoni and precipitin tests, and 29 of them gave positive complement-fixation tests; the sera of two of the animals were highly anticomplementary. Hydatid cysts of the liver and lungs were found in each of the 31 immunologically positive sheep.

The series of patients suffering from hydatid disease who have been studied by means of the purified antigen is too small to permit of any sweeping conclusions, but the data indicate that the antigen is sufficiently sensitive, and the increased uniformity of the quality of the antigen eliminates a very undesirable source of variation.

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THE ANAEROBIC GLYCOGEN CONSUMPTION IN ASCARIS FEMALES AND MALES*

THEODOR V. BRAND

Our knowledge of whether in ascarids the differences in the morphology of the sexes are combined with differences in chemical composition and metabolism, is very poor. Fauré-Fremiet (1913) states that one compound of the ether extract, the ascarylalcohol, is only found in females. Toryu (1932) finds that the females of *Parascaris equorum* (*Ascaris megalocephala*) store more glycogen than the males. He states that in starvation experiments the former can use 64 per cent and the latter 75 per cent of the stored glycogen and that the anaerobic CO_2 output is more marked in the females. Under aerobic conditions the gaseous exchange is more pronounced in males than in females, both in *Ascaris lumbricoides* and *Parascaris equorum* (Harwood and Brown, 1934; Toryu, 1934). The present investigation has been undertaken in order to solve some questions concerning the carbohydrate metabolism of both sexes in *Ascaris lumbricoides* from the pig.

As Ascaris is deprived of oxygen most of the time under natural conditions (v. Brand and Weise, 1932), the experiments have been performed under anaerobic conditions (hydrogen stream passing through the container). The worms were freshly collected for each experiment from the slaughterhouse. They were washed with saline, rapidly wiped dry with filter paper, weighed and kept in 1.1 per cent NaCl solution in an incubator of 37 ± 1° C. In longer lasting experiments the saline was changed every 24 hours. Analyses according to Pflüger's glycogen method were performed each time on groups of 10 animals (occasionally with males on groups of 7–8) at the beginning and after 24, 48 or 72 hours of starvation. The percentage of glycogen was always calculated on the basis of the weight of the worms at the beginning of the experiment in order to avoid errors owing to changes of the weight during the experiments. It was, therefore, not possible to extend the experiments over a longer period than 72 hours as after 96 hours some of the worms were always dead and the accurate weight of the remaining living worms was of course not known. All lots, in which an animal had died, were discarded. A worm was regarded as dead when it did not move spontaneously or after mechanical stimulation. The average weight of the females was 4.14 ± 0.09 gm. (extremes: 5.09 to 2.52 gm. in 550 worms),

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that of the males 1.13 ± 0.03 gm. (extremes: 1.62 to 0.67 gm. in 468 worms).

The mean glycogen content of worms analyzed as soon as possible after having been removed from the pigs was relatively similar in both sexes. The females had stored 5.29 ± 0.28 per cent glycogen of the weight of the living worms, the males 5.77 ± 0.35 per cent. These figures are derived from 20 parallel determinations on both sexes each time on worms collected at the same time, but without certainty that they lived in the same individual pigs. The difference in the sexes in this series is not statistically significant. But in view of the fact that from 20 experiments 15 showed a more or less higher glycogen content in the males, it is possible that the males really store a little more glycogen than the females and certainly not less as reported by Toryu (1932) for *Parascaris equorum*. It should be noted that the glycogen content of the females in the present studies is definitely lower than found previously (v. Brand, 1934) in Denmark (5.29 to 7.18 per cent), but about the same as reported for ascarids analyzed in Germany, where Weinland (1900, 1901) found 5.4 per cent of glycogen. It would be of interest to investigate whether such differences are connected with different feeding of the pigs in different countries or if they are merely due to different treatment before slaughtering.

In order to know the rate of glycogen consumption the material can be treated in two ways: (1). It is possible to calculate the glycogen-consumption for the single experiments by subtracting the percentage of glycogen found in the starvation lots from that of the worms analyzed at the beginning of the same individual experiment and to form from the figures obtained in this way the mean values. This has been done in the third column of table 1. (2). It is possible to form first the mean values of the glycogen content of the different lots from all experiments and to calculate the glycogen consumption by subtracting the figures found in the starvation lots from that calculated for the worms analyzed at the beginning. This calculation (column 4 of table 1) should give the same values as the first, if it had been possible to analyze in the single experiment each time worms of both sexes at the beginning and after the three starvation periods, or if the glycogen consumption would have been uniform in all experiments. This was, however, not the case. The glycogen consumption varied in the different experiments in relatively wide limits and it was, in the single experiment, often only possible to analyze worms at the beginning and after one or two of the starvation periods instead after each of the three periods. But the figures obtained by the second calculation are in approximate agreement to those found by the first. It seems reasonable to assume that the most probable values will be the mean values of the two above calculations (column 5 of table 1). The

TABLE I.—*Glycogen consumption and content, in grams per 100 grams of fresh worms, of* Ascaris lumbricoides, *males and females, during starvation under anaerobic conditions at 37° C.*

Time		Number of experiments	Total glycogen consumption			Daily glycogen consumption	Glycogen content from which is computed the total glycogen consumption in method II (see text)
			Method I	Method II	Average of methods I and II		
Beginning of experiment	Females	20					5.29 ± 0.28 (3.08 – 7.80)
	Males	20					5.77 ± 0.35 (3.18 – 8.85)
24 hrs.	Females	11	1.44 ± 0.15 (0.98 – 2.43)*	1.21	1.32	0–24 hrs. 1.32	4.08 ± 0.34 (3.06 – 6.65)
	Males	11	1.36 ± 0.18 (0.64 – 2.21)	1.36	1.36	1.36	4.41 ± 0.43 (2.48 – 7.12)
48 hrs.	Females	12	2.40 ± 0.21 (0.94 – 3.45)	2.37	2.38	24–48 hrs. 1.06	2.92 ± 0.29 (1.08 – 4.46)
	Males	10	3.08 ± 0.24 (1.71 – 4.54)	2.83	2.95	1.59	2.94 ± 0.41 (1.11 – 4.76)
72 hrs.	Females	11	3.18 ± 0.23 (2.35 – 4.38)	2.93	3.05	48–72 hrs. 0.67	2.36 ± 0.19 (1.54 – 3.62)
	Males	9	4.19 ± 0.33 (2.67 – 5.27)	3.72	3.95	1.00	2.05 ± 0.26 (0.51 – 3.03)

* Figures in parentheses are the extremes in each series.

glycogen consumption on the single days of starvation are derived from these figures and are summarized in column 6 of table 1.

It is obvious that during the first day of starvation the rate of glycogen consumption is identical in both sexes, or at least that the differences are so small that they cannot be demonstrated in this series. The curve of glycogen consumption during the three first days of starvation is somewhat different in both cases. In the females the glycogen consumption rate decreases from the beginning until the end. It corresponds on the second and third days to 80 and 51 per cent of the first day rate. Whereas the males consume during the second day even some glycogen more than on the first and the decrease begins only on the third day, where a figure of 74 per cent of the first day rate is found. These differences are probably not due to experimental errors, since they find a reasonable explanation in the behavior of the worms. The females are very active only during the first day, but later on quiet, whereas the males remain active throughout the greater part of the experiments and quiet down only during the third day. It is not surprising that the glycogen consumption rate in both sexes is very similar since the energy required for life can be derived under anaerobic conditions almost exclusively from carbohydrates. Sexual differences in relative consumption rate of carbohydrates, fats and protein, which may occur in free living organisms, are not very likely in the case of an anaerobic living organism.

It is finally of interest to compare the above figures with those found by Toryu (1935) for *Parascaris equorum*. The glycogen content is here much lower: females 3.15 and in another series 3.8 per cent, males 2.75 and 2.9 per cent. Practically the whole anaerobic glycogen consumption takes place here during the first day in the case of the female (consumption of the first day 1.97 gm. for 100 gm. worms, the second 0.18 gm. only), whereas in the male the corresponding figures are 1.32 and 0.73 gm. The amount of material used by Toryu was relatively small and it is possible that further work will show that the similarities to *Ascaris lumbricoides* are still greater.

It should be emphasized that the amount of glycogen present in the ascarids can apparently vary within a wide range without being the limiting factor for glycogen consumption. *Ascaris lumbricoides* females in the United States with 5.29 per cent consume during the first day 1.32 gm., in Denmark with 7.18 per cent glycogen, 1.39 gm. and *Parascaris equorum* females with 2.75 per cent consume 1.97 gm. glycogen. This is a characteristic difference between parasitic nematodes and many free living animals. It is known that a mammal which has stored much glycogen has a high respiratory quotient and that its level declines with the progressive depletion of the glycogen stores during starvation. This indicates that the share of the glycogen in the total metabolism decreases.

Amongst the free living worms it has been demonstrated (v. Brand, 1936) that in planarians the rate of carbohydrate consumption is definitely higher in animals containing much polysaccharide than in those of the same species which have stored only small amounts. This is of course due to the fact that in the metabolism of free living organisms the glycogen can be replaced easily by other substances from which energy can be derived, as for example fat. Whereas in the case of anaerobic living parasites it is, according to our present knowledge, doubtful whether this is at all possible.

SUMMARY

The glycogen content of the female of *Ascaris lumbricoides* from the pig is a little lower than that of the male. The glycogen consumption is identical in both sexes during the first 24 hours of starvation, but later on it decreases more rapidly in the female. The glycogen consumption seems to be much more independent of the glycogen content of the body than in free living organisms.

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AN INCREASE IN THE PROPORTION OF BASOPHILIC LEUCOCYTES IN GUINEA PIGS EXPERIMENTALLY INFECTED WITH SWINE LUNGWORMS

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INTRODUCTION

The experimental demonstration by Schwartz and Alicata (1934) that *Metastrongylus elongatus,* a nematode parasitic in the lungs of swine, could develop to maturity, though not to egg-laying maturity, in guinea pigs suggested further studies on this rather unusual host-parasite relationship. It is the purpose of this paper to report the blood cell reaction of the experimental host and to call particular attention to the marked increase in the percentage of circulating basophilic leucocytes or basophiles in guinea pigs experimentally infected with lungworms.

The life cycle of this parasite as determined by Hobmaier and Hobmaier (1929) and Schwartz and Alicata (1934) is briefly as follows: Embryonated eggs passed in the feces of infested swine are swallowed by earthworms with the feces and the larvae develop to the infective third stage principally in the wall of the esophagus. When earthworms containing the third stage larvae are swallowed by swine or when larvae removed from this intermediate host are given to guinea pigs or other susceptible hosts, the larvae penetrate the hosts' intestinal wall and follow the course of the lymph and ultimately reach the lungs. Here, or in the lymph glands, the larvae undergo two moults as early as the third day after entering the host, and maturity is reached in about a month. The development of these worms in the lungs is known to produce a verminous bronchopneumonia which may result in extensive consolidation and emphysema. A cough and respiratory disturbances are the common symptoms. These symptoms and gross lesions in the lungs have been described in guinea pigs by Schwartz and Alicata.

MATERIALS AND METHODS

The infective larvae fed to guinea pigs were obtained from naturally infested earthworms collected in hog lots at Leonardtown and Bethesda, Maryland. The most common earthworm found in the manure in hog lots in the above-noted localities was the brandling or stinking earthworm, *Helodrilus foetidus;* this earthworm was easily kept in moist earth in the laboratory to supply larvae as needed. To procure the larvae, the esophagus was removed from the earthworm and teased apart with dissecting needles in normal physiologic saline. As most of these intermediate

hosts harbored large numbers of infective larvae, a plentiful supply was readily available. The larvae were counted and definite numbers were fed to guinea pigs with a pipette. No attempt was made to determine the species of lungworm larvae at the time of experimental infection, but when worms were discovered post mortem they were all specimens of *M. elongatus*.

Young guinea pigs weighing between 175 and 275 grams and known to be free from helminths were used in this study. The guinea pigs were fed a diet of oats supplemented once a week with cabbage.

Differential counts of 100 white blood cells were made on coverslip smears, stained with Wright's blood stain. Blood for this purpose was obtained from the animal's ears, smears being prepared in the early afternoon of the days on which observations were made.

Histological studies were made on sections of lung tissue fixed in 10 per cent formalin and stained with azure II—eosin and hematoxylin.

EXPERIMENTAL DATA

In figures 1–3 the percentages of basophiles, neutrophiles and lymphocytes are recorded in the form of graphs for four experimental animals and one control animal on various days throughout the duration of the experiments. The proportion of eosinophiles and monocytes fluctuated within normal limits and the data were not plotted. Detailed information regarding the white cell counts on individual guinea pigs follow.

EXPERIMENTAL ANIMALS

Guinea Pig 6. Differential leucocyte counts were made on this animal at frequent intervals as noted in the graph, from September 12, 1935, to January 28, 1936 (fig. 1). During this time the guinea pig was fed infective lungworm larvae on three different occasions as noted elsewhere in this paragraph. A peak of 25 per cent basophiles was reached on October 2, 16 days after the first infection with 300 larvae on September 16. The proportion of the basophiles dropped off rapidly at first and then more slowly until a 2 per cent level was reached by October 14, 28 days after infection. On October 25, a second infection of 400 larvae was given and the proportion of basophiles reached 47 per cent on November 11, 17 days later. There was then a gradual decrease of these cellular elements to 4 per cent on December 6, 42 days after the infection. A third infection of 600 larvae was given on December 6 and this was followed by another increase in the basophiles. This time the basophile count was 32 per cent 7 days after the infection but returned to the 2 per cent level on January 4, 29 days after the larvae were given.

An increase in the proportion of lymphocytes and a decrease in the proportion of neutrophiles accompanied the rise in basophiles after the

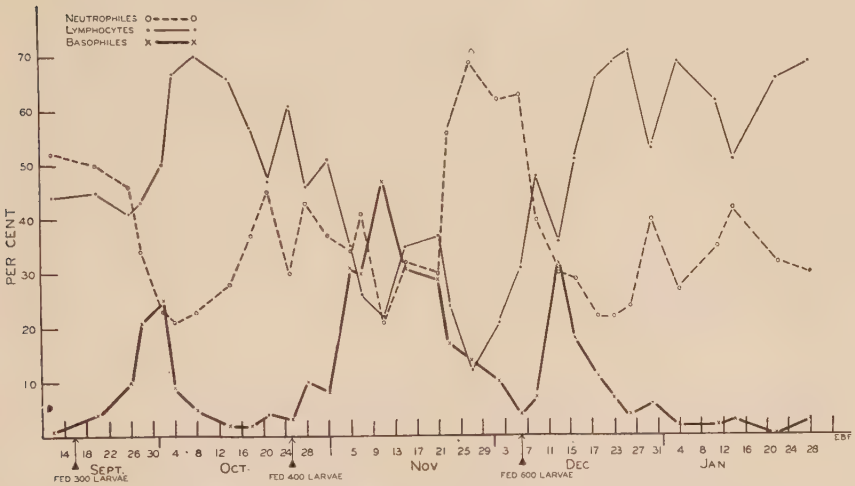

Fig. 1. Percentages of basophiles, neutrophiles, and lymphocytes observed in guinea pig 6 which received three experimental infections with swine lungworms.

first infection. As the percentage of basophiles increased following the second infection the percentage of lymphocytes and neutrophiles decreased, but after the peak of basophiles was passed the proportion of neutrophiles increased while that of the lymphocytes decreased, the exact opposite of the picture following the first infection. After the third infection there was an increase in lymphocytes with a corresponding drop in neutrophiles similar to that observed after the first infection.

The symptoms observed in this guinea pig were labored breathing, a cough and a rough coat. These symptoms appeared 10 days after the first dose of larvae was fed and lasted for the next 15 days. The symptoms were most noticeable during the time the basophile count was high and diminished as the proportion of these cells returned to normal. More severe respiratory difficulty was observed from the eleventh to the twenty-ninth day after the second infection. During this time there was a loss in weight of 50 grams and at times the animal was so weak it could hardly move. The third infection was followed by less severe symptoms. Slight pulmonary embarrassment was noticeable, however, as late as 53 days after the final infection, the date when the animal was killed.

At necropsy the lungs showed some hemmorhage and a few small emphysematous areas on the ventral surface. No lungworms were recovered from the lungs, but microscopical examination revealed evidence of their having been present. There was considerable inflammation in the interstitial tissue and particularly around the bronchi, the infiltration consisting largely of eosinophiles and neutrophiles. Some areas of the lung were consolidated while others showed vesicular emphysema. Ex-

tensive fibrosis in the septal tissue throughout the lung was noted; this was probably the result of the earlier infestations.

Guinea Pig 7. This animal was observed parallel with guinea pig 6. On September 16, 150 infective lungworm larvae were fed. On September 28 and October 2, respectively, 12 and 16 days later, the proportion of basophiles was 9 per cent (fig. 2). After a count of 2 per cent on

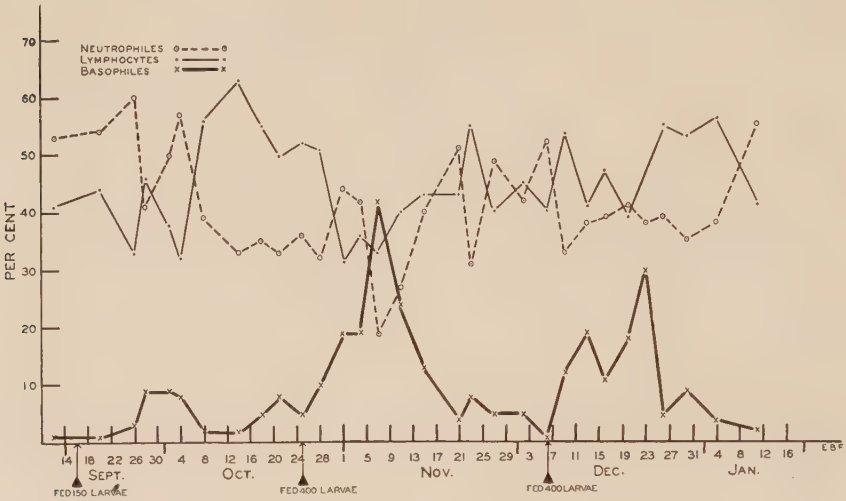

Fig. 2. Percentages of basophiles, neutrophiles, and lymphocytes observed in guinea pig 7 which received three experimental infections with swine lungworms.

October 8 and 14, the proportion of basophiles rose to 8 per cent on October 21 and then decreased to 5 per cent on October 25, on which date 400 more lungworm larvae were fed. This was followed by an increase in the proportion of basophiles until a count of 42 per cent was recorded on November 7, 13 days later. When the count was down to 1 per cent on December 6, 42 days after the second infection, a third dose of 400 larvae was given. Seven days after this feeding the basophile count rose to 19 per cent; a higher peak of 30 per cent was observed on December 23, 17 days after infection, after which there was a decline until a count of 2 per cent was made on January 11, 36 days after the larvae were given.

The proportion of lymphocytes and neutrophiles varied from 32 to 63 per cent and 19 to 60 per cent, respectively, during the period of observation. Following the first infection, there was an increase in lymphocytes and a decrease in neutrophiles. The second infection was followed by a decrease in the proportions of lymphocytes and neutrophiles, after which the percentages of these two cellular types fluctuated within the normal range until observations were stopped 36 days after the third infection.

Ten days after the first dose of larvae was given symptoms appeared and lasted for about 12 days. The weight of the animal dropped from 198 grams to 175 grams in the first six days; after this there was a gradual gain in weight. At the time the second infection was given, one month after the first infection, the animal weighed 200 grams. On November 1, a week after the second infection, symptoms again appeared and while they were not as severe as in the first infection, they lasted for 20 days. The animal gained weight during this period and when it was infected for the third time it weighed 235 grams. Difficulty in breathing appeared 6 days after the third dose of larvae was fed and this persisted until the animal died on January 14, 33 days later. Both lungs showed hepatized areas at necropsy but no worms were recovered. Histologically the lung tissue resembled that in pig 6, except for the presence of considerable hemorrhagic congestion. Associated with this were numerous alveolar phagocytic cells containing blood pigment.

Guinea Pig 12. Differential white cell counts were made on this animal from November 7 to December 26, parallel with pigs 6 and 7. This guinea pig was fed 600 lungworm larvae on December 6, when the above-named animals were infected for the third time. The proportion of basophiles which had varied between 1 and 4 per cent until this time, began to increase until a peak of 46 per cent was reached on December 23, 17 days after infection and five days before death (fig. 3, A). The increase in the proportion of basophiles was accompanied by a decrease in the proportion of neurophiles and lymphocytes.

On December 13, 7 days after the larvae were fed, this animal began to show symptoms of lungworm infestation; the symptoms increased in

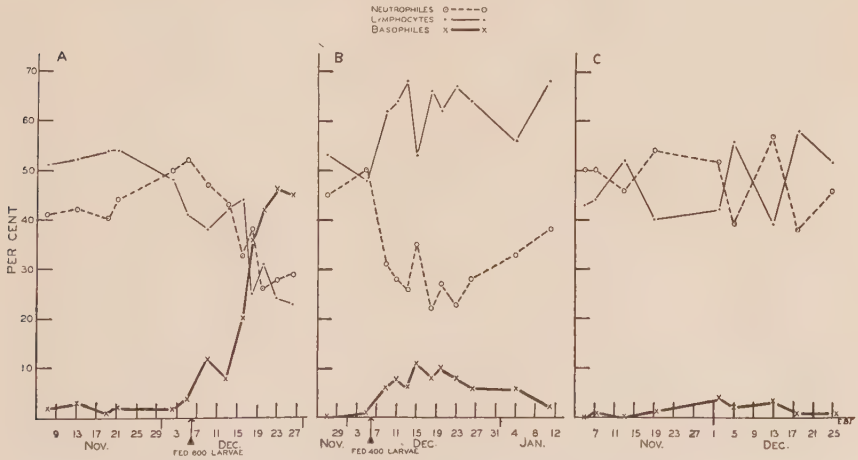

Fig. 3. Percentages of basophiles, neutrophiles, and lymphocytes observed in: A, guinea pig 12 experimentally infected with swine lungworms; B, guinea pig 13 experimentally infected with swine lungworms; C, guinea pig 11, uninfected control.

severity until the fourteenth day when the host animal was scarcely able to breathe. The condition of the guinea pig became steadily worse until death on December 28, 22 days after infection. The basophile count two days before death was 45 per cent. At necropsy the lungs were extensively hepatized and the bronchi contained worms and an exudate. Nineteen specimens of *Metastrongylus elongatus* which had reached maturity, though not egg-laying maturity, were recovered from the bronchi. Part of one lobe of the lung taken for sectioning also contained an undetermined number of worms. Acute broncho-pneumonia and bronchitis were diagnosed from sections of the lung. The bronchial epithelium showed marked hyperplasia and desquamation especially in areas where worms were present. The exudate in the bronchi contained many neutrophilic and eosinophilic leucocytes. These cells as well as lymphocytes and macrophages were observed in considerable numbers in the pulmonary tissue, particularly around the bronchi. The lymphocytic infiltration, however, was not as extensive in this animal or in any of the guinea pigs involved in these experiments as has been described for infested swine by Nieberle (1924) and others. One striking difference between the histology of the lungs of this guinea pig and those previously discussed was the presence in the former of numerous basophiles in the peribronchial tissue. Unlike the eosinophiles, which were even more plentiful, the basophiles were not seen migrating through the broncial wall nor were they observed in the exudate in the lumen of the broncial tubes.

Guinea Pig 13. The white cell picture of this animal was studied parallel with studies on the blood of the other experimental animals, from November 27 to January 11 (fig. 3, B). This guinea pig was fed 400 infective lungworm larvae on December 6, the date on which pigs 6 and 7 were infected for the third time and pig 12 was infected for the first time. The proportion of basophiles rose to a peak of 11 per cent on December 15, 9 days after infection, after which there was a gradual decrease to 2 per cent on January 11, 36 days after infection. The rise in the proportion of basophiles was accompanied by an increase in the proportion of lymphocytes and a corresponding decrease in proportion of neutrophiles. The proportion of neutrophiles increased as the basophiles returned to normal.

Difficulty in breathing was noticed 7 days after infection and this became more pronounced by December 20, 14 days after infection. The basophiles were then at the 10 per cent level. The host animal showed some improvement after January 4 but died on January 13, 38 days after infection. No worms were found in the lungs at post-mortem examination. Sections of the lungs showed extensive hemorrhagic congestion with edema. The cellular infiltration in connection with the infammatory changes was similar to that observed in guinea pig 12, with the excep-

tion that fewer eosinophiles were present. Basophiles were not observed in the pulmonary tissue.

CONTROL ANIMALS

Differential counts of white blood cells were made on several uninfected animals at various times during this study. The proportion of the different types of cells were approximately within the normal range for guinea pigs as given by Scarborough (1930) whose data were compiled from 829 counts made by 34 observers. Whereas the normal proportion of basophiles, according to Scarborough, is from 0 to 2 per cent (mean 0.77 per cent), the present author occasionally found the proportion of basophiles in the circulatory blood of guinea pigs to be as high as 4 per cent in apparently normal animals.

For the sake of comparison with the infected animals, the percentages of neutrophiles, lymphocytes and basophiles is one control animal (pig 11) are plotted in figure 3, C. These counts were made at intervals as shown in the graph, from November 5 to December 26, coincident with most of the counts made on the infected guinea pigs during the same period. Post mortem examinations of guinea pig 11 as well as of two other uninfected controls showed no abnormalities. Histological examination of the lungs revealed a normal picture. Few eosinophiles and no basophiles were found in the lungs of these uninfected controls.

DISCUSSION

From the cases described in this paper it is evident that the increase in the percentage of basophiles is the most constant change in the white blood cell picture of guinea pigs following each infection with lungworms. This increase appears to be correlated with the presence of the worms in the lungs. The first differential counts made 3 days after each infection showed an increase in the percentage of basophiles with the exception of the counts after the first infection in the case of guinea pig 7. In this case only 150 larvae were fed; the basophiles did not increase until 10 days after infection. In the experimental animals that survived until the basophile count returned to normal, the proportion of these cells was higher than normal for a period of 28 to 42 days after infection, with the peak coming between the seventh and seventeenth days; this corresponded approximately to the presence of the most severe symptoms.

In their experiments on guinea pigs, Schwartz and Alicata observed lungworm larvae with the sheaths of the two final molts, in the lungs 3 days after infection. Fifth-stage larvae and young adults, recognizable as *Choerostrongylus pudendotectus* and *Metastrongylus elongata,* were found by these writers 11 days after infection, and well developed specimens of *M. elongatus* were recovered by them in two cases, in one case

28 days and in the other 31 days after infection. In one of their experiments in which five guinea pigs were infected at the same time, one host animal harbored several mature specimens of *M. elongatus* 24 days after infection but no worms were found in the other four animals at necropsy 30 days after the infection. From their description of the gross pathological lesions in the lungs of the latter host animals, it appears that worms had been present but were eliminated in some manner before death.

In the experience of the author, mature specimens of *M. elongatus* were found in one guinea pig 22 days after infection. In this case (guinea pig 12) the proportion of basophiles was 45 per cent two days before necropsy and microscopical examination revealed a large number of basophile in the lungs. On the other hand no worms were found in guinea pigs 6 and 7, necropsied 53 and 39 days, respectively, after the third infection, nor in pig. 13, necropsied 38 days after the first infection. Before death, the proportion of basophiles in the circulating blood of these animals was from 2 to 3 per cent and these cells were not found in histological sections of the lungs. It was evident from the pathological picture of the lungs and from the symptoms shown by the host animals in question when the basophile count was high, that lungworms had been present. From these observations it appears probable that the proportion of basophiles is directly correlated with the presence of the worms in the lungs, that is, the percentage of basophiles increases as the worms develop and decreases when or soon after the worms disappear from the lungs.

Although further investigations are necessary before any definite conclusions can be drawn from the data presented in this paper, there is some evidence that the number of larvae administered or previous infections might influence the increase in proportion of basophiles. In pig 6 (fig. 1) the proportion of basophiles rose to 25 per cent after 300 larvae were fed while a peak of only 9 per cent was reached after 150 larvae were fed to pig. 7 (fig. 2). An examination of the graphs for these two animals shows that the resulting proportion of basophiles was practically the same after 400 larvae were given to each animal as a second infection. It is not known whether the previous infections had any effect on the increase in the basophiles in these animals since the white cell picture of a guinea pig receiving a first infection with the same number of larvae was not studied by the writer parallel with the observations on guinea pigs 6 and 7. The third infections of pig 7 (400 larvae) and pig 6 (600 larvae) was followed by a lower proportion of basophiles in each case than that resulting from the previous infections of 400 larvae; the proportion of basophiles in pig 12 (fig. 3, A), given 600 larvae as a first infection on the same date that pigs 6 and 7 received the third infection was higher than in either of the latter two animals. The indication that cases of reinfection may show a lower proportion of basophiles than ob-

served after primary infections meets conflicting evidence, however, in the case of pig 13 (fig. 3, B). In this animal the proportion of basophiles reached a peak of only 11 per cent following an initial infection with 400 larvae, this being much lower than the proportion of basophiles observed in pigs 6 and 7 following the third infection.

Since there are a large number of parasitic infestations in different hosts in which a generalized and local eosinophilia have been reported, the appearance of an increase in number of basophiles in the circulating blood and in the lungs, in conjunction with a local eosinophilia is quite striking. Kennedy and Law (1935) have demonstrated a basophilic leucocytosis in foxes infected with *Toxascaris leonia;* the resulting increase in the number of basophiles appeared to be directly proportional to the number of infective ascarid eggs fed. Since Kennedy (1933) did not find cells distinguishable as eosinophiles but did find cells distinctly basic in staining in the blood of foxes, Kennedy and Law concluded that in cases involving an increase in the number of basophilic cells in foxes, the eosinophiles are replaced by the latter blood elements. The eosinophiles and basophiles exist as distinct cells in the blood of guinea pigs, however, and in response to some parasitic infestations this host animal reacts in the classical manner by showing an increase in the proportion of eosinophilic leucocytes in the blood. It has been known for some time that an eosinophilia is present in guinea pigs experimentally infected with *Trichinella spiralis* (Opie, 1904). The present author has also observed an eosinophilia from 14 to 23 per cent with an occasional increase in the basophiles to 5 or 6 per cent in cases of experimental trichinosis and experimental pulmonary ascariasis (*Ascaris suum*) in guinea pigs. The increase in the proportion of basophiles in these cases is of doubtful significance, however, since basophiles at a level of 4 per cent were observed by the writer in uninfected guinea pigs. Since an eosinophilia is observed in guinea pigs infected with some other metazoan parasites, the increase in basophiles is, apparently, a response to the invasion of lungworms. This suggests the possibility that in guinea pigs the basophiles may under certain conditions, as for example in experimental lungworm infection, play a rôle similar to that of eosinophiles.

SUMMARY

Differential leucocyte counts were made on guinea pigs experimentally infected with undetermined species of swine lungworms. It was observed that the most constant change in the white blood cell picture following each infection was an increase in the proportion of basophiles, the basophile count running as high as 46 to 47 per cent. Large numbers of basophilic leucocytes were also seen in the cellular infiltration into the lungs of one guinea pig (No. 12) harboring *Metastrongylus elongatus,*

22 days after infection, the proportion of these cells in the circulating blood being 45 per cent two days before death. Basophiles were not seen in the lungs of guinea pigs which had not been infected or in guinea pigs that had apparently lost their infection, and presumably, the proportion of circulating basophiles had returned to normal following the loss of the worms.

The data presented in this paper indicate that the increase in the percentage of basophiles is probably related to the development of the worms in the lungs, that is, basophiles first appear when the worms reach the lungs, increase in number as the worms develop, and finally return to normal when or after the parasites apparently leave the lungs. Some evidence is also presented which suggests that the proportion of basophiles may be directly proportional to the number of larvae given and that the percentage of these cells may not increase following reinfection to the extent they increase following an initial infection.

Since a generalized eosinophilia, as observed in several helminthic diseases, also occur in the guinea pig when infected with some metazoan parasites other than lungworms, the increase in the percentage of basophiles as recorded in this paper, is believed to be a peculiar cellular reaction of the guinea pig to the invasion of the lungs by swine lungworms. The writer is of the opinion that in the cases presented in this paper the basophile may play a rôle similar to that usually accorded to the eosinophile.

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THE STABILITY OF THE CAT AND DOG STRAINS OF *ANCYLOSTOMA CANINUM**

A. O. Foster and W. W. Cort

When investigations upon hookworm disease in dogs were begun by this laboratory in 1925, a strain of *Ancylostoma caninum* Erc. was obtained for experimental use from a naturally infected dog in Baltimore. The first of a long series of studies upon the biology of this parasite was made by Herrick (1928) and this was followed closely by Scott's (1928) study of the development of this species in normal and abnormal hosts. Among other results of these studies was the observation that this strain of the dog hookworm was able to reach sexual maturity in *both* cats and dogs, but that the former was a highly refractory host in which, at best, only a small percentage of a given dose of infective larvae was able to survive.

Meanwhile (Sept., 1925), a cat obtained from Cold Spring Harbor, Long Island, was autopsied and found to harbor 10 specimens of *A. caninum*. Larvae were cultured from the contents of the rectum and administered to a young dog, in which host they showed "a peculiar lack of infectivity" (Scott, 1929, p. 209). On the other hand, this strain was readily perpetuated in cats. In subsequent experiments, Scott (1929, 1929a) was able to demonstrate that he was dealing in these cases with physiologically different strains which were especially adapted to the dog and to the cat.

An evaluation of the data of this report necessitates a summary consideration of the quantitative infectivity of these strains in cat and dog hosts. The behavior of the dog strain in dogs has been especially well studied during years of experimental hookworm work in this laboratory, yet it has seemed advisable for the present purpose to consider only those data which were obtained under exactly parallel conditions, where, for example, larvae from the same culture were tested at the same time in both hosts. Such data have been presented by Scott (1929), McCoy (1931), and Foster and Cort (1932), and, in passing over the individual results obtained, it should be stated that the findings by each author were essentially the same. The summarized comparative data upon the infectivity of these strains is as follows, where the percentages represent the average proportion of infective larvae which reached maturity in young

* From the Department of Helminthology of the School of Hygiene and Public Health of the Johns Hopkins University and the Gorgas Memorial Laboratory. Panama, R. P. This work was made possible through the cooperation of the International Health Division of the Rockefeller Foundation.

hosts, and the figures in parentheses are the number of animals which were tested:

	In dogs	In cats
Dog strain	55.0% (9)	2.3% (21)
Cat strain	0.5% (16)	33.0% (21)

It has seemed from these data that the dog strain is better adapted to the dog host than is the cat strain to the cat host, and that cats in general are more susceptible to infection with the dog strain than are dogs with the cat strain. But the most important conclusion is that both strains are highly infective to their respective hosts and only slightly so to the opposite host.

Perhaps the most significant observation upon the stability of these strains was made by Scott (1930). He noted that the dog strain retained its infectivity for dogs and did not become more infective to cats, even after three successive generations in the cat host. On the other hand, in a single experiment where the cat strain was passed through the dog host, a higher infectivity was obtained in one dog (20 per cent) than in one cat (5 per cent). This apparently reversed infectivity of the cat strain, after a single generation in the dog host, caused both Scott (1930a, p. 89) and McCoy (1931, p. 200) to lament the absence of further information upon that point, since its confirmation would lead to many interesting considerations with respect to several other parasites of both medical and veterinary importance. These were the reasons for making the observations contained in this report. The same physiological strains of *A. caninum* were employed and relatively young animals were used throughout.

OBSERVATIONS AND DATA

Over a period of about two years, the cat strain was passed through nine successive generations of dog and cat hosts, and its infectivity noted at each stage. The data and host passages are presented in Table 1 and Chart 1.

As a starting point, infective larvae were cultured from eggs passed by D 642, which had been infected by McCoy (1931, p. 197) with 6,600 larvae of the straight cat strain. It became positive to Lane examinations in 17 days and was autopsied on the 34th day. Six worms were recovered, giving a development of 0.1 per cent, which is very typical of the development of this strain in dogs.

Larvae cultured from eggs passed by D 642 were given to 2 dogs and 1 cat (Table 1, A). Both dogs began passing eggs after periods of 15

TABLE I.—*Infectivity of the cat strain of* A. caninum *after varying numbers of passages through dog and cat hosts*

Larvae from	Given to	Days, infection to autopsy	Larvae given	Worms recovered	% development	
					in dogs	in cats
		A. Infectivity after 1 generation in a dog				
D 642	D 646	31	532	430	80.80	
	C 472(a)	31	1,600	0		0.00
	D 652	28	266	82	30.90	
		B. Infectivity after 2 generations in dogs				
D 646	D 658	28	280	134	48.00	
	C 480(a)	25	20,000	0		0.00
	C 484(b)	7	20,000	700(?)		3.50
D 652	C 483	52	20,000	107		0.53
	C 479	28	20,000	19		0.09
	D 665	29	200	149	74.50	
		C. Infectivity after 3 generations in dogs				
D 665	D 664	Not autopsied	325	—	—	
	C 496(b)	49	7,200	2		0.03
	C 497(a)	49	7,200	0		0.00
	C 512	47	6,000	5		0.08
		C_1. Infectivity after 2 generations in dogs and 1 in a cat				
C 483	D 666	15	2,000	918	45.50	
	D 695(b)	12	1,000	870	87.00	
	C 508(a)	21	2,000	997		49.85
	C 514	54	1,000	5		0.50
	C 511	54	500	9		1.80
		D. Infectivity after 2 generations in dogs and 2 in cats				
C 511	C 513(b)(d)	51	685	1		0.15
	C 518(c)(d)	156	1,000	0		0.00
	C 519(d)	40	1,350	152		11.26
	C 520(d)	19	1,350	244		18.10
	D 687	40	700	243	34.80	
		E. Infectivity after 2 generations in dogs and 4 in cats				
C 518	C 516(a)(d)	21	11,000	0		0.00
	C 523(a)(d)	21	1,000	0		0.00
	C 524(a)	21	11,000	0		0.00
	C 525(a)	21	1,000	0		0.00
	C 534	82	1,000	8		0.80
	C 535(c)	61	1,000	0		0.00
	C 536(b)	19	750	80		10.70
	C 537(b)	10	750	180		24.00
		F. Infectivity after 2 generations in dogs and 4 in cats				
C 534	C 550	165	7,000	5		0.07
	C 551	31	7,000	57		0.81
	C 552	32	7,000	28		0.40
		G. Infectivity after 2 generations in dogs and 5 in cats				
C 550	C 570	121	2,020	337		16.68
	C 573	29	1,720	638		37.09
		H. Infectivity after 2 generations in dogs and 6 in cats				
C 570	C 577(b)	27	2,000	998		50.00
	C 579(b)	28	2,000	1,100		55.00
	C 553(b)	12	77,000	2,600		3.38
	D 728(a)	32	2,000	0	0.00	

(a) Negative throughout to Lane examinations and negative at autopsy.
(b) Negative throughout to Lane examinations but worms present at autopsy.
(c) Positive to Lane examinations earlier, but worms lost before autopsy.
(d) Starved 2–2½ weeks during interval between infection and autopsy.

and 18 days, respectively, while the cat remained negative. At autopsy on the 28th day, one dog (D 652) yielded 82 worms, this representing about 31 per cent development. The other two animals were autopsied 3 days later, and no worms had matured in the cat, while in the dog (D 646) a better development (81 per cent) obtained than has usually

occurred in instances where the straight dog strain has been used. This repetition of Scott's (1930, p. 151, Table 1, D) experiment, therefore, gave even stronger support for his original hypothesis than did his own data. It does appear, then, that the cat strain loses in infectivity for cats and becomes highly infective to dogs (simulating the straight dog strain) after passage through the dog host. Scott's figure of 5 per cent for the development of this strain in one cat, under similar conditions, is probably high, although it need not be accounted for by individual variation in host-susceptibility, since this cat was autopsied only 20 days after infection, and as shall be clear from the data to follow there is a great loss of worms from the more refractory hosts just before sexual maturity is reached.

For the next generation, the strain was continued from each dog into 2 cats and 1 dog (Table 1, B). More cats were employed as well as larger larval doses, in order to insure, if possible, the resumption of the strain from the cat host. Both dogs (D 658 and D 665) developed good

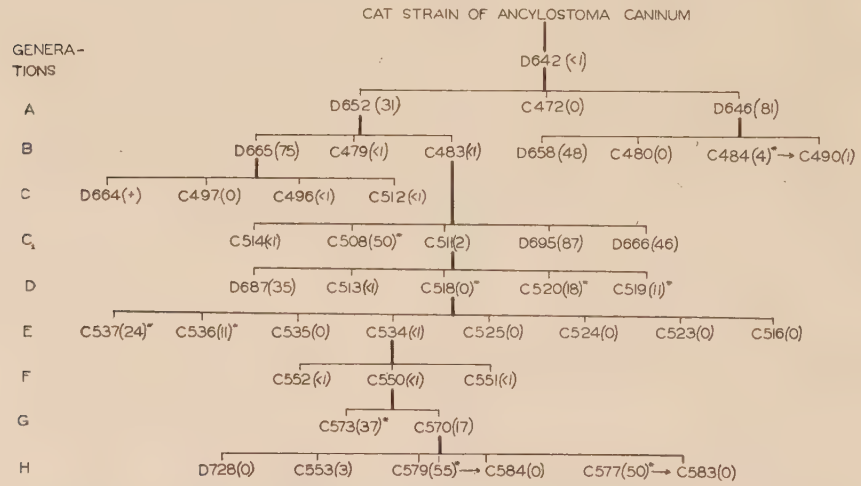

CHART 1. Experimental host passages, beginning with the straight cat strain of *A. caninum*. After the number of each animal is given the percentage development of hookworms based upon the number recovered at autopsy. Stars following these percentages indicate instances where the figures are usually high or low as a result of factors which are explained in the text or in Table 1.

infestations after prepatent periods of 14 and 15 days, respectively, and at autopsy, four weeks after infection, it was found that 48 and 75 per cent of the larvae given had matured. Of the 4 cats, one remained negative 25 days and no worms were found at autopsy. Two others, C 479 and C 483, showed developments of 0.09 and 0.53 per cent. The fourth cat died on the 7th day after infection, and about 700 young worms were recovered from the intestine. Although this represented 3.5 per cent of

the number of larvae given (20,000), this figure cannot be regarded as a measure of the infectivity of this strain, since the worms had not matured. The young worms were transferred by gelatin capsule to an uninfected cat (C 490) which, at autopsy 17 days later, yielded 10 worms (1.43 per cent development of the transferred larvae). On the basis of the original larval dose given to C 484, the percentage development to the 24th day in the "secondary" host was about 0.05 per cent. It has been our experience, as it was Scott's (1930, p. 157), that attempts to establish infestations by transferring "half-grown" worms are usually unsuccessful. For this reason, we attach some interest to the above result.

In Table 1, C, are given the data upon the infectivity of the cat strain after one additional generation in dogs. Of this series D 664 had a considerable infestation as shown by a high egg count although the percentage development was not known since the animal was not autopsied. It is quite clear that the infectivity during this and the previous generation was essentially similar to that of the straight dog strain.

In Table 1, C_1, are given the data on a strain which is exactly comparable with that of Table 1, C, except for the last host passage. For this generation the strain was continued through a cat (C 483) after 2 host passages in dogs (see Table 1, B). The behavior of the strain in this generation was looked upon as very important because this originally cat-infective strain had reversed its infectivity after one generation in the dog host, while, on the other hand, it had been observed that the dog strain did not become more infective to cats for at least 3 generations. As far as could be determined from the data, the cat strain had reverted to the dog strain type during the period of our experimental observations. The data have not been easy to interpret. In the first place 3 of the 5 animals were apparently killed by doses of less than 2,000 larvae. Among these animals were the two dogs (D 666, D 695) and one cat (C 508), and their percentages of development are probably exaggerated because, except for D 666 the worms had not yet reached sexual maturity at the time of autopsy. The findings on the other two cats have influenced strongly our interpretation of the findings upon this generation. When both were autopsied, 54 days after infection, it was found that in these hosts, 0.5 and 1.8 per cent of the larvae given had matured. These figures approximate what would have been anticipated had larvae of the straight dog strain been given. Therefore, it is not clear that the infectivity for dogs had diminished nor that the infectivity for cats had increased as a result of the last passage through the cat.

To test the infectivity of the cat strain after two generations in dogs and two in cats (D 642–D 652–C 483–C 511), larvae were cultured from C 511 and administered to 4 cats and 1 dog (Table 1, D). In this

instance varying numbers of larvae, in smaller doses, were given and, in addition, the 4 cats were starved for intervals of 14 to 18 days in an attempt to introduce a factor which might (possibly) render them more susceptible to infection. All 5 animals developed infestations, as was determined either by Lane examinations or by recovery of worms at autopsy. Cat 518 showed a prepatent period of 28 days (as did C 519 also) but was not autopsied until the 156th day since it was used as a source of culture to infect subsequent generations. At autopsy, however, no worms were recovered, a result which indicates a short-lived infestation and a consequent inadaptibility between host and parasite. One cat and the dog (C 519 and D 687) were autopsied on the 40th day and yielded developments respectively of 12 per cent and 35 per cent. Cat 520, which had been autopsied still earlier (19 days) showed a development of 18 per cent. It is thus apparent that in comparing the infectivity of these strains, the age of the infestation at the time of autopsy is a factor, since these data and other contained in this report, indicate that in partially refractory hosts the infestations are lost quite rapidly. The development in D 687 (35 per cent), although somewhat lower than the *average* for the straight dog strain, must be accepted as within the range of infectivity. On the other hand, the developments in C 519 and C 520 were significantly higher than has been obtained with the straight dog strain in cats, although these figures have been approached when the cat hosts have been maintained on a deficient diet (Foster and Cort, 1932). It must be concluded that these two cats were abnormally susceptible either as a result of the influence of starvation upon their resistance or in consequence of a favorable change in the infectivity of the strain as a result of the last generation in the cat host. The data of the next generation indicate that starvation was probably the influential factor, even though the starved animals did not become uniformly less resistant.

The strain was continued from C 518 into 8 cats (Table 1, E). One cat died on the 10th day (C 537) and another on the 19th (C 536), although the causes of death are not clear. In these cases all of the worms were still immature, and as has been indicated from previous experiments there were *relatively* large numbers of worms present at autopsy. These, however, as in the earlier cases, cannot be accepted as criteria of the infectivity of the strain. This is further borne out by the fact that on the 21st day 4 additional cats, two of which, in this case also, had been starved, were all negative at autopsy. These findings emphasize again the loss of infestation which takes place in the refractory host just before the parasites reach sexual maturity. Of the two remaining cats, one (C 534) began passing eggs on the 23rd day and was kept as a culture source for the next generation. The other (C 535) was positive to Lane examination on the 25th day, but failed to produce more than

4,000 E. P. D.[1] and soon went negative again. At autopsy no worms were found, an observation which again indicates a short patency in the refractory host. Cat 534 was autopsied on the 82nd day and 8 worms were recovered, representing a development of 0.8 per cent.

From C 534, the strain was continued into the next generation which consisted of 3 cats (Table 1, F). These all began passing eggs on the 24th day, and soon reached egg outputs of from 17,000 to 36,000 E. P. D. Two were autopsied four weeks after infection and showed developments of 0.4 and 0.8 per cent respectively. The other (C 550) was maintained for 165 days and continued to produce about 15,000 E. P. D. At autopsy only 5 worms were recovered (0.07 per cent devel.), which may indicate that part of the infestation had been lost.

Meanwhile, two cats were given larvae cultured from eggs passed by C 550 (Table 1, G). Cat 573 of this generation was given 1,720 larvae and began passing eggs 26 days after infection. Three days later it died, apparently as a result of parasitism, as it was very anemic and 638 worms were recovered. This was equivalent to a 37 per cent development. The other cat (C 570) began passing eggs on the 34th day and reached a maximum of 381,000 E. P. D. on the 66th day after infection. It was not autopsied until the 121st day when 337 worms were recovered. In this case a dose of 2,020 larvae had been given, with the result that the development, after 87 days of patency, was about 17 per cent. In this generation, although the prepatent periods were relatively long and not at all uniform, there was an unexpected degree of infectivity in the cat host.[2]

In view of this result it seemed especially important to observe the infectivity of this strain in an additional generation of cats, and even more important to test again its infectivity for dogs. Therefore, three cats and one dog were given larvae from C 570 (Table 1, H). One cat (C 553) was given an especially large dose of larvae (77,000) and died 12 days after infection, at which time about 2,600 (3.4 per cent development) young worms were recovered from the intestine. The other two cats had been given only 2,000 larvae each, but died on the 27th and 28th days after infection, without having become positive to Lane examina-

[1] E.P.D. = Eggs per day.

[2] It is desired to acknowledge that this result, inasmuch as it pertains to the 6th generation in cats, contradicts the last sentence of a footnote by Foster and Daengsvang (1932, p. 246) which referred to this work when it was in progress. At that time the autopsy results of this generation were not known, although the behavior during the 5 previous generations and the long prepatent periods in the sixth, seemed at that time, to justify the statement that this strain had apparently not increased in infectivity for cats during six generations in that host. In the light of the data presented at this time, it may be seen that the earlier conclusion was incorrect, since, by autopsy findings, there was apparently an increased infectivity for the cat manifested for the first time in the sixth generation.

tions. From C 577 were recovered 998 worms (50 per cent) and from C 579, 1,100 (55 per cent). Meanwhile the one dog included in this generation had not yet begun passing eggs, with the result that any continued study of this strain appeared to depend upon the successful transfer of worms from C 577 and C 579 to uninfected cats. Therefore, 100 worms from each host were administered by gelatine capsule to C 583 and C 584. Then on the 32nd day, D 723 was autopsied but no worms were found. It is unfortunate that the importance of testing this strain in more dogs had not been foreseen, and especially so, in view of the fact that the worm-transfers to C 583 and C 584 (autopsied shortly afterwards) failed to establish infestations. Consequently, after having passed the cat strain through two generations in dogs followed by six successive generations in cats, it was impossible to continue the experiments further. It is clear, however, that the findings of this last generation confirmed the recently increased infectivity of this strain for the cat host, and, because of the negative result on one dog, there was an indication of reduced infectivity for the dog host. That this strain produced fatal infestations in cats from doses of only 2,000 larvae is strong evidence of its increased ability to develop in that host. Yet, since the worms did not reach sexual maturity at the time of autopsy, it is probable that the apparent infectivity of this strain is exaggerated in the figures given upon percentage development (Table 1, H). Moreover, the extended prepatent periods (over 27 days) suggest a doubt that this strain had yet reached the condition of adaptability to the cat which is exhibited by the straight cat strain.

DISCUSSION

The available evidence seems to indicate that the cat strain is the less stable or the "weaker" of the two strains. It is less widely distributed in nature (Scott, 1929) and as a rule cats are not so frequently infected nor are their infestations as large as in dogs. It has an inherently lower infectivity for the cat host than has the dog strain for the dog (refs. cit.), and also a lower viability of eggs and larvae (Foster and Daengsvang, 1932). The cat strain also develops less well in dogs than does the dog strain in cats. Moreover, it exhibits in the normal host a slower growth rate and reaches a smaller adult size (Scott, 1929 b, 1929 c) and has a lower average egg production per female worm (McCoy, 1931). Growth and egg output, however, do not appear to be inherently controlled functions of the worms but seem rather to be regulated by the host. When infestations of the cat strain were established in dogs, Scott noted that the worms matured as rapidly and reached as large a final size as did worms of the dog strain in this host. The egg production per female worm was found by McCoy to be about the same for both strains in the

cat (about 2,400 E. P. D.) and this figure was multiplied several times in the case of infestations of either strain in the dog host.

The present findings confirm Scott's (1930) observation that the infectivity of the cat strain is reversed after a single passage through the dog host. Moreover, it seemed in the present study, that once this change had taken place, the strain behaved like the dog strain, retaining its infectivity for the dog during several successive generations in cats. After the fifth generation, however, the strain appeared to be regaining its infectivity for cats, although the data are insufficient to establish definitely this apparent evolution of strains.

These results point also to a deficiency in our knowledge of the stability of the straight dog strain, since the effect upon this strain of as many as seven successive generations in cat hosts has not been determined. It does not appear, however, that there is anything in our present findings to contradict the view that the dog strain is older biologically and perhaps ancestral to the cat strain. This view, in turn seems to suggest that the evolution and experimental behavior of these strains might be explained by simple biological selection. The difficulty with this hypothesis, however, is that it lacks adequate experimental testing and is not easily harmonized with certain experimental findings, such, for example, as the increased development of these parasites in starved hosts or those on deficient diets. Also in experimental infections of susceptible normal hosts some of the larvae which fail to develop are found to be viable and infective to other susceptible hosts.

Besides having suggested experimentally an apparent evolution of these strains, this study has thrown some additional light upon the biology of the dog hookworm. It has been indicated, for example, that starvation may render a refractory host less resistant to parasitism, an effect which is comparable to that of a deficient diet (Foster and Cort, 1932). Also the behavior of *A. caninum* in partially abnormal hosts has been observed with interest because of its possible bearing upon the mechanism or operation of host resistance. The refractory condition manifests itself in a longer prepatent period, shorter patent period, decreased egg output of the infestation, and in a lower percentage development. Moreover, in the partially refractory host a significant proportion of the infestation is eliminated just before the worms reach sexual maturity. It seems possible that this phenomenon may be associated with the difficulty of establishing infestations by the transfer of "half-grown" worms. This is perhaps an indication that the infective larvae have a greater facility of orientation than have worms whose development is already initiated.

A. caninum is a common parasite of cats and dogs throughout the temperate and tropical world, and the relationships between the strains normally infecting these hosts seem to be as discussed above. This para-

site has occurred, however, in many other hosts such as the jackal, wolf, fox, yagouroundi, tiger, sloth-bear, and raccoon. One of the present authors (A. O. F.) has identified *A. caninum* from the Central American puma[3] (*Felis bangsi costaricensis* Merriam) which appears to be an unrecorded host for this species. He was also fortunate to have observed two interesting hookworm infestations in four-week-old Panama yagouroundis (*Herpailurus yagouroundi panamensis* (Allen)). These were litter mates which died after a short period in captivity although they ate well and should have thrived. At autopsy mixed infestations of *A. caninum* and *A. pluridentatum* were found. One harbored 17 specimens of the former and 59 of the latter species, while the other yielded 15 and 94 specimens respectively. The deaths were attributed to hookworm infestation. This shows that *A. caninum* may occur naturally in many wild relatives of cats and dogs and that in some of the observed cases the infestations have been large enough to suggest that they may be, perhaps, something more than examples of accidental parasitism. It would be of interest to test the infectivity of some of these wild strains in cats and dogs, for comparison with the two physiological strains which are especially adapted to these hosts.

ABSTRACT SUMMARY

Continuing an experimental inquiry into the stability of the two physiological strains of *Ancylostoma caninum* which was begun by Scott in 1929, data were obtained upon the behavior of the cat strain during two generations in dogs followed by seven successive generations in cats. Scott's observation that the cat strain took on the infectivity of the dog strain after passage through the dog host was confirmed. It was found further that, when this change had occurred, the strain remained highly infective to dogs and only slightly so to cats for several successive generations in cat hosts. From the fifth to the seventh generations, however, the strain seemed to regain in part its infectivity for cats. It was thought that this might constitute an experimental evolution of these strains, and it was concluded that the dog strain was more stable than the cat strain and probably ancestral to it.

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RESEARCH NOTES

A NEW TYPE OF ALLOCREADIID CERCARIA: THE CERCARIAE OF *ANALLOCREADIUM* AND *MICROCREADIUM*

During the past summer a study of the life cycles of *Anallocreadium armatum* (MacCallum 1895) and *Microcreadium parvum* Simer 1929 was begun, but it has been interrupted by the press of other work. I am publishing descriptions of the cercariae now because of the light they throw on some puzzling problems in taxonomy.

The two cercariae discussed here were obtained from the snail *Amnicola peracuta* Pilsbry & Walker in the Little Brazos River, Brazos County, Texas. Of 248 *A. peracuta* examined, 10 contained the *Anallocreadium* cercaria and 6 contained the *Microcreadium* cercaria. Both cercariae have: (1) cuticular spines, (2) setae or "sensitive hairs" covering the sides of the body, (3) three pairs of setae on the tail, (4) pigmented eyespots, (5) a long slender tail, and (6) no stylet. The second characteristic distinguishes them from the trichocercous cercariae of *Lepocreadium* and *Pharyngora,* the fifth distinguishes them from the microcercous or cotylocercous cercariae of *Plagioporus, Hamacreadium* and *Sphaerostoma,* and they differ from the ophthalmoxiphidiocercaria of Allocreadium and Crepidostomum in the first, second, third, and fifth characteristics. This new type of cercaria, which may be called the Anallocreadine type, may be defined as follows: Distome cercariae with straight slender tail, undivided sac-like excretory bladder, pigmented eyespots, cuticular spines, no stylet, numerous setae on sides of body, and three pairs of setae on tail; developing in rediae in freshwater snails.

The cercaria of *Anallocreadium armatum* (Fig. 2)

Setae on sides of body from tip to tip, but those near ends usually soon lost. Cuticular spines present, sometimes limited to anterior end. Numerous oil droplets (up to 20 μ in diameter) scattered through body from level of eyespots to posterior end. Dorsal pigmented eyespot on each side of prepharynx. Three pairs cephalic glands anterior to ventral sucker, staining red in neutral red. Oral sucker slightly smaller than ventral sucker. Oral opening subterminal, prepharynx longer than pharynx, esophagus shorter than pharynx, crura running nearly to posterior tip of body. Testes tandem, anterior to excretory bladder. Excretory bladder an elongated oblong or rectangular sac reaching two-thirds to three-fourths way to ventral sucker, bladder-wall cells filled with light-refractive droplets which form dark-brown mass in life and stain red in neutral red; collecting tubes leaving bladder on each side of anterior tip, dividing at level of ventral sucker; six flame cell groups on each side of body, most anterior group having 8 flame cells. Tail, extended, longer than body and tapering to sharp tip; contracted, much shorter than body, with bluntly rounded tip; very narrow dorsal and ventral fins, not always visible; 3 pairs of setae on papillae about midway of length, usually soon lost in swimming. Swimming continuous and vigorous, with body strongly contracted and flexed ventrad, tail extended and beating very rapidly in "figure 8" fashion. Positively phototaxic when dissected out of snail but negatively phototaxic after emerging spontaneously. Measurements (killed by heat, unflattened, in water): Body, length 0.40 to 0.45 mm.; width 0.13 to 0.18 mm.; tail, length 0.24 to 0.32 mm., width 0.04 to 0.05 mm.; oral sucker, length 60 to 70 μ, width 40 to 65 μ; ventral sucker, length 60 to 80 μ, width 60 to 80 μ; pharynx, length 27 to 33 μ, width 25 to 33 μ; eyespot, diameter 18 to 22 μ; setae, length 15 to 20 μ.

This may be *Cercaria thalia* Faust 1921, but Faust's description differs from my specimens in several important points, most notably in the lack of setae in *C. thalia.* However, Faust's "agamodistomum of *Cercaria thalia*" is certainly an

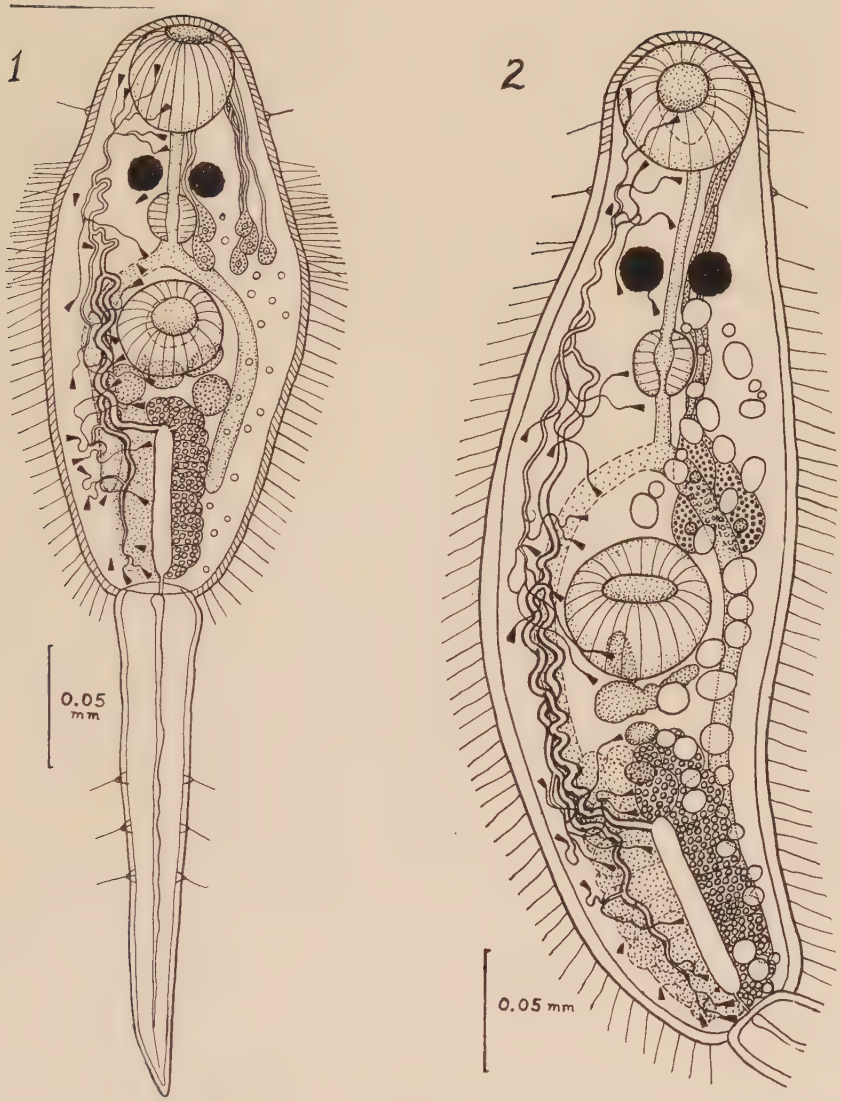

FIG. 1. Cercaria of *Microcreadium parvum*, ventral view, composite camera drawing of heat-killed specimens with details added from living specimens; excretory system omitted on left side, gland cells omitted on right.

FIG. 2. Cercaria of *Anallocreadium armatum*, ventral view, composite camera drawing of heat-killed specimens with details added from living specimens; excretory system omitted on left side, gland cells omitted on right.

Anallocreadium. My description of the cercaria of *Anallocreadium armatum* is based entirely on the ten lots of cercaria referred to in my notes as the "Large form"; in addition to these, I have found an *Anallocreadium* cercaria in two *A. peracuta* which consistently differs from the "large form" in having a much smaller body, fewer and smaller oil droplets in body, and non-refractive droplets

in bladder-wall cells, but this form has not been determined experimentally and may turn out to be a variant of the same species.

The cercaria of *Microcreadium parvum* (Fig. 1)

Setae on sides of body from posterior end to level of eyespots or farther forward, thickly clustered between levels of ventral sucker and eyespots. Cuticular spines covering entire body. Body nearly clear, containing only 20 to 40 small (5 μ) oil droplets. Dorsal pigmented eyespot on each side of prepharynx. Six pairs indistinct cephalic gland cells anterior to ventral sucker, which do not stain in neutral red. Oral sucker slightly larger than ventral sucker. Oral opening subterminal or terminal, prepharynx longer than pharynx, esophagus shorter than pharynx, crura ending about halfway between testes and posterior end of body. Testes larger than eyespots, placed symmetrically one on each side of anterior tip of bladder. Excretory bladder sausage-shaped, reaching to level of testes; bladder-wall cells containing colorless non-refractive droplets which stain red in neutral red; collecting tubes leaving each side of anterior tip of bladder, dividing near level of anterior edge of ventral sucker; six flame cell groups on each side, flame cell formula apparently $2[(6+6+6)+(6+6+6)]$. Tail, extended, longer than body, tapering to sharp tip; contracted, much shorter than body, rounded at tip; narrow dorsal and ventral fins running most of length of tail; three pairs of seta-bearing papillae about midway of tail, setae usually persisting. Swimming and light reactions as in *Anallocreadium armatum* cercaria. Measurements (killed by heat, unflattened, in water): Body, length 0.230 to 0.280 mm., width 0.110 to 0.130 mm.; tail, length 0.200 to 0.240 mm., width 0.035 mm.; oral sucker, length 42 to 50 μ, width 42 to 45 μ; ventral sucker, length 38 to 45 μ, width 43 to 47 μ; pharynx, length 20 μ, width 20 μ; eyespots, diameter 15 μ; setae, length 15 to 20 μ.

The cercaria of *A. armatum* and the cercaria of *M. parvum* both begin their development in stout sausage-shaped rediae about 1.0 mm. long which have a very large pharynx 60 to 120 μ long and a nearly globular intestine containing globules of orange pigment. The rediae develop in the liver of the snail. The cercariae leave the rediae at a very early stage of development and migrate to the lymph spaces, where they become mature. The cercariae of the two species differ in number of cephalic glands, size, relative proportions of the suckers, and in several minor features, but the most striking difference is the position of the testes, which are tandem in *A. armatum* and symmetrical in *M. parvum*.

My identification of these two cercariae is based on the morphology of metacercariae obtained by experimental infestations. Twenty specimens of *Musculium ferrissi* were obtained from one spot in the Navasota River; ten of these were dissected and found to be uninfested; five were exposed to swarming cercariae of *Microcreadium parvum*, all of which became infested; five were exposed to cercariae of *Anallocreadium armatum* and three became infested. The experimentally infested Musculium were dissected at intervals ranging from one to fifteen days. The older metacercariae obtained in this way in each case resemble the respective adults so closely as to leave very little doubt of their identity. No change could be seen in *Microcreadium* metacercariae after the seventh day. *Anallocreadium* metacercariae evidently continue growth and development much longer; 15 days after encystment they were about 0.6 mm. long, but many found in natural infestations were 1.0 to 1.5 mm. long, had nearly mature vitellaria, and could hardly be distinguished from adults of *A. armatum* except for the lack of eggs in the uterus. These experiments will be continued next summer if time permits.

Stunkard (1932; Parasitology, 24: 321–343) and Hopkins (1934; Ill. Biol. Monogr., 13: 49–124) have commented on the very different types of cercariae reported for various members of the family Allocreadiidae; Stunkard suggests that this family is a heterogeneous group and should be divided. Manter (1936;

Carnegie Inst., Publ. No. 457: 33–38) remarks "The genera *Anallocreadium* and *Crassicutis* show affinities to the Lepocreadiinae through the genus *Homalometron* . . . Further evidence of relationship to the Lepocreadiinae is to be sought in life history studies." The Anallocreadine type of cercaria does show some resemblance to the cercariae of the Lepocradiinae, but in some respects it resembles also the cercariae of *Crepidostomum* and the cercariae of *Plagioporus;* perhaps the Anallocreadine type is the nearest thing we have to an intermediate type connecting the several dissimilar cercariae of the Allocreadiidae.—Sewell H. Hopkins, *A. and M. College of Texas, College Station, Texas.*

ISOSPORA VOLKI N. SP., A NEW AVIAN COCCIDIAN

In 1933 the writer (1933a, Zeitschrift f. Parasitenk. 6: 147–149) gave a brief account of a coccidial infection in a single, captive six-plumed bird of paradise, *Parotia lawesi lawesi* Ramsay. The coccidian involved appeared to be an undescribed *Isospora,* but a specific name was not proposed because the observations were limited to a single living host in which the asexual stages could not be studied. Recent observations on similar infections in a number of additional hosts, together with the fact that studies on the tissue stages must be postponed indefinitely, due to the rarity of hosts, seem to justify the description of this form as a new species at this time. *Isospora volki* n. sp. is the name proposed, in honor of Mr. Joseph Volk, who first observed the oocysts in December, 1929.

The following recent findings are of significance: Oocysts apparently identical with those previously described from one specimen of *Parotia lawesi lawesi* have now been found in the feces of two additional specimens of the same sub-species; oocysts indistinguishable morphologically from the above have been found in the feces of several other birds belonging to the same family; the single host previously examined, in the Milwaukee Zoo, was originally of this same lot of birds and was brought to this country from New Guinea at the same time (information through the courtesy of Mr. Crandall); this single host has now shown oocysts for over six years, the latest positive record, kindly supplied by Mr. Volk, being that for January 13, 1936.

These facts may be resolved as follows. Several captive birds from the same original habitat exhibit chronic infections due to coccidia the oocysts of which, as a group, can be distinguished morphologically from those described from other birds. An isolated host has maintained a similar infection for several years. It seems logical to suppose that the infections were naturally acquired and that the coccidia involved are natural parasites.

A diurnal periodicity in the appearance of the oocysts was observed in these infections. This is apparently characteristic of many Isosporan infections in birds (Boughton, 1933b, Amer. Jour. Hyg. 18: 161–184); note of it has already been made in the case of the first bird of paradise examined.

Specific Diagnosis

Systematic position. Isospora volki n. sp. (Coccidiida, Eimeriidae).

Description. Oocysts: discharged in unsegmented stage (Fig. 1, a.); broadly ovoid at first; wall delicate, tending to collapse around sporocysts after sporulation (Fig. 1, c.); no micropyle evident; measurements on 50 oocysts as follows: range of length 14–21 μ; mean length 18 μ; range of width 14–20 μ; mean width 16 μ; sporulation complete within 48 hours in feces at room temperature; a ring-shaped globule often present between sporocysts.

Sporocysts: ellipsoid, with "plug-like" structure at the more pointed pole; wall thin but firm; poles of the two sporocysts usually pointed in opposite directions; sporocysts irregularly arranged around a granular sporocystic residue.

Hosts. Type host: Parotia lawesi lawesi Ramsay (Aves Passeriformes Paradisiedae). The parasite presumably lives in the intestinal epithelium, although no tissue stages have been observed. Morphologically similar oocysts were

FIG. 1. Oocysts of *Isospora volki* n. sp. a. Unsegmented oocyst. b. Sporoblast stage. c. Sporulated oocyst.

observed in fecal samples from the following birds belonging to the family Paradisiedae: *Paradisaea apoda apoda* L., *P. apoda salvadori* Mayr and Rand, *P. guilelmi* Cabanis, *P. minor minor* Shaw, *Paradisornis rudolphi rudolphi* Finsch, *Epimachus meyeri meyeri* Finsch, *Seleucides melanoleucus melanoleucus* (Daudin), *Laphorina superba minor* Ramsay, *Uranornis rubra* (Daudin), *Manucodia chalybatus orientalis* Salvadori.

From the Department of Animal and Plant Pathology, the Rockefeller Institute for Medical Research, Princeton, N. J. The writer is indebted to Mr. Lee S. Crandall, Curator of Birds, New York Zoological Gardens, for permission to examine a number of rare birds.—DONALD C. BOUGHTON.

SUCCESSFUL INFECTION OF MICE WITH *STRONGYLOIDES RATTI*

Sandground (1925, Jour. Parasit. 22: 59–80) reported unsuccessful attempts to infect the following animals with fairly large numbers of larvae of *S. ratti*, young animals being used for the purpose: four rabbits, two guinea pigs, and six mice. He suggested the possibility that the prolonged passage of this parasite through rats may have produced a rat-restricted population which might account for its inability to establish itself in an unusual host, even though this may be closely related zoologically to the normal host.

The writer was able to establish infections in four out of 12 mice. The mice were each inoculated subcutaneously with 500 filariform larvae and autopsied eight days later. By use of the autopsy technique described elsewhere (Sheldon, 1937, Amer. Jour. Hyg. 25: 53–65) fifteen, five, five and one adults respectively were recovered from the intestines of four of the twelve mice. The remaining eight were negative. Cultures made from the feces of the four infected mice contained only small numbers of larvae. This experiment suggests the possibility of developing a mouse strain of *Strongyloides ratti* by repeated passage through mice.—A. J. SHELDON, *Department of Helminthology, School of Hygiene and Public Health, Johns Hopkins University.*

A NEW SPECIES OF *URANOTAENIA* (DIPTERA, CULICIDAE) FROM HAITI

Among some mosquitoes recently received from Dr. S. S. Cook, Port-au-Prince, Haiti, were several specimens of an undescribed species of *Uranotaenia*, which is of especial interest because in the male sex the first tarsal segment of the fore legs is extremely short, only about one-fourth as long as the second tarsal segment.

So far as I know, no other American species of the true mosquitoes (subfamily Culicinae) exhibits such a peculiarity, although short basitarsal joints of

much the same sort are found in all the legs of both sexes of the species of *Corethra* (Mochlonyx) of the subfamily Chaoborinae. Similar modifications of the fore legs of the male have been reported in two Oriental (*U. metatarsata* Edw. and *U. atra* Theo.) and one African (*U. pallidocephala* Theo.) species of *Uranotaenia,* but in the two latter species, at least, the first fore tarsal is said to be about half as long as the second.

In the present species, although the first fore tarsal segment of the male is so short, the second, third and fourth segments are unusually long, so that the entire tarsus is not much shorter than usual. Expressing the lengths of the tarsal segments as percentages of the length of the whole tarsus, the female of the new species has the fore tarsal "formula": 42+27+17+9+5=100. This agrees fairly well with similar measurements for both sexes of several other American species of *Uranotaenia.* However, the fore tarsus of the male of the new species gives the following figures: 10+41+27+16+6=100, showing the extreme reduction of the first joint and the lengthening of the next three segments. I propose to give this species the name of *U. cooki,* in honor of its collector.

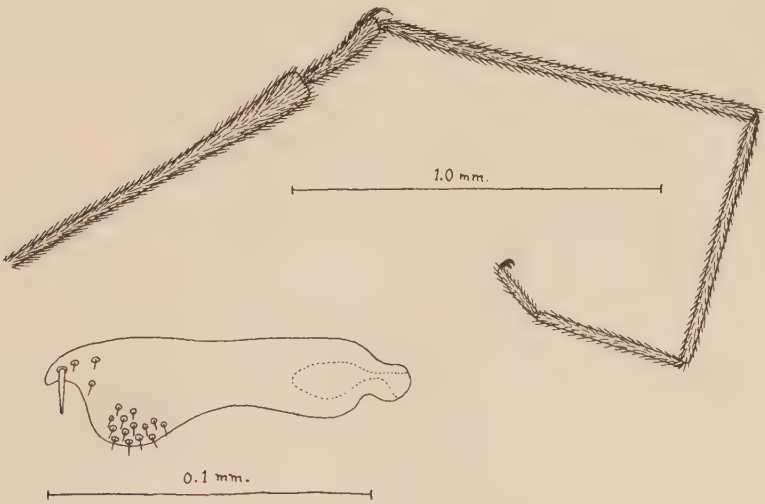

FIG. 1. *Uranotaenia cooki* new species. Fore tarsus of male and clasper of male hypopygium.

Diagnosis—A small brownish species agreeing in coloration with *U. pallidoventer* Theo., except for the presence of conspicuous lateral white spots on abdominal segments three to seven. The male differs from all described American species of the genus in the great reduction in length of the first tarsal segment of the fore legs.

Female. Proboscis black, longer than the abdomen, slightly swollen at tip. Palpi very short, black. Antennae dark with sparse whorls of short hairs. Occiput covered mainly with flat black scales, among which are a few erect forked ones. A broad stripe of bluish-white scales bordering the eyes. Thoracic integument yellowish brown. Prothoracic lobes covered with flat bluish-white scales. Mesonotum and scutellum with scattered black scales. Just in front of the wing base on each side is a blackish integumental spot, across which runs a broad stripe of bluish-white scales, extending from the wing base less than halfway to the anterior border of the mesonotum. In the middle of the sternopleura is another blackish integumental spot, also crossed by a short broad stripe of bluish-white scales. A small crescent-shaped patch of bluish-white scales at the base

of each coxa. Abdomen black above with conspicuous apico-lateral patches of bluish-white scales on segments three to seven. Venter yellowish white. Wings with black scales, especially dense on costa, subcosta and first vein. Bases of first and fifth veins with short stripes of bluish-white scales. Legs dark, tips of femora and tibiae with small patches of bluish-white scales dorsally, these patches larger on hind legs. Femora yellowish below, the middle pair broad and flat toward base. Length about 3 mm.

Male. Coloration, proboscis and palpi as in the female. Antennae dark with sparse whorls of long hairs. First tarsal segment of fore legs very short, about one-seventh as long as tibia and one-fourth as long as second tarsal segment. Second, third and fourth fore tarsal segments unusually long. Hypopygium much like that of *U. pallidoventer* Theo., but the claspers shorter and stouter and much swollen just before the tip.

Described from two males and three females, captured by Dr. S. S. Cook in a ravine near his house in Port-au-Prince, Haiti, on Feb. 11 and Apr. 12, 1932. Dr. Cook describes the locality as follows in his letter: "Flood waters erode the banks of the stream and leave jutting ledges of rock. In such places there are small pools of quiet water, constantly shaded and without vegetation, except for rotting leaves. The adults rest on the under surface of the rocks and fly when disturbed, but immediately return to the dark hiding place. I have not been able to induce them to bite. Larvae are very scarce in these pools."

The type male and female have been deposited in the U. S. National Museum, the other specimens are retained in the collection of the Johns Hopkins School of Hygiene and Public Health.—Francis Metcalf Root.

From the Department of Protozoology and Medical Entomology, School of Hygiene and Public Health, Johns Hopkins University. The manuscript of this article was found among Dr. Root's papers after his death.

A SIMPLE METHOD OF STAINING AND MOUNTING NEMATODE LARVAE

While studying the changes guinea-worm embryos undergo in the body cavity of cyclops, various methods of staining and mounting these embryos at different stages of development were tried, of which the following one gave the best results.

The solutions required for this method of staining and mounting are:

1. *Fixative:*	Mercuric perchloride	8 grs. (0.52 gms.)
	Sodium chloride	16 " (1.04 gms.)
	Distilled water	100 cc.
2. *Stain:*	Methylene blue (saturated alcoholic solution diluted to times with distilled water	2 cc.
3.	Giemsa stain (concentrated stock solution, from Kasauli)	12 cc.
	Liquor ammonia fortis	1 drop
	Liquor ammonia fortis	1 drop
	Distilled water	50 cc.
4. *Preservative:*	Formalin solution	2%

4. *Sealing material:* Gold-size and Apathy's cement.

Method of Staining and Mounting

1. About 5 to 10 specimens of infected cyclops, containing guinea-worm larvae undergoing developmental changes, are isolated in a drop of distilled water on a perfectly clean slide. The specimens are brought to the center of the

slide and after draining the water, by means of small bits of filter paper, 2 or 3 drops of the fixative solution are placed on the specimens. The cyclops die in this solution in about one to two minutes whereas the guinea-worm larvae take from 20 to 30 minutes to die. The cyclops are carefully dissected and the guinea-worm larvae are gently released from the body cavities. The dissection of dead cyclops before the guinea-worm larvae die facilitates to a large extent the release of the larvae without injury.

2. While the larvae are still actively moving in the fixative solution a thin coverslip (No. '0', size 2″×⅞″), is placed on the slide and the fixative solution is allowed to spread evenly under the coverslip. Care must be taken that the solution does not spread out of the coverslip area. After keeping the larvae thus for from 3 to 5 minutes the slide is gently warmed over a spirit lamp until small bubbles of gas just begin to appear under the coverslip. This amount of heat will be just sufficient to kill the larva in a fully stretched condition.

3. The slide is then cooled and examined under the microscope. After noting the exact position of the larvae, a few drops of the stain are added, (on that side of the coverslip which is nearest to the larvae), and allowed to spread evenly while gradually drawing out the fixative solution by means of filter paper from the other sides of the coverslip. In doing this, the larvae are best placed in position under the low power of the microscope so that care may be taken to see that they do not move out of the coverslip area; this may be done by adjusting the position and the force with which the fixative fluid is drawn out by the filter paper. A few drops of stain are then placed at one end of the coverslip to replace the stain that evaporates and to prevent drying of the preparation. The slide is then kept under a Petri dish for about 24 hours. In warm weather about 2 cc. of water is kept in a small glass cup under the Petri dish to prevent too rapid evaporation. The gradual concentration of the stain helps considerably in the differential staining of the larvae.

The exact time for which the larvae should be kept in the stain depends on the particular larvae to be studied. For guinea-worm larvae 24 hours are necessary, whereas for *Paracamellanus* larvae 12 hours are quite sufficient. It has however been noticed that the larvae do not usually become overstained, even when they are kept in contact with the stain for a longer period than necessary. If they are overstained they can be easily destained to the required depth by washing them with 0.2 per cent hydrochloric acid.

4. The slide is again examined under the microscope and, after noting the exact position of the larvae, a few drops of formalin solution are added to that side of the coverslip which is nearest to the larvae. The stain is then gently washed out by draining it away by means of small bits of filter paper. This washing requires care, patience and frequent checking of the position of the larvae under the coverslip. By means of light pressure on the coverslip, movement of the coverslip itself, and adjustment of the force and direction of the flow of the formalin solution, it is quite possible to keep control of the position of the larvae.

5. The stained larvae are finally mounted in 2.0 per cent formalin solution and the coverslip is sealed on all sides with gold size and, when this has dried, with Apathy's cement. As a measure of further safety, another coat of gold size is applied on top of the Apathy's cement to prevent any possibility of leakage of the preservative fluid. This method of sealing makes the slide absolutely watertight and specimens mounted by this method have been preserved in a tolerably good condition for the past six years.

Discussion

This method of staining and mounting has been adopted for staining guinea-worm larvae and *Paracamallanus* larvae at various stages of their development in the body cavity of cyclops and also for nematode larvae recovered from the intestinal contents of fish. It seems likely that other nematode larvae can also easily be stained and preserved. By the same method, dissected parts of copepods (like

cyclops, diaptomus and daphnia), have also been stained. These, unlike the nematode larvae, take the stain better if they are treated with 0.4 per cent hydrochloric acid just before the addition of the stain. The specimens should, however, be washed several times with distilled water to remove the acid thoroughly before the addition of the stain.

When large quantities of larvae are available, as for instance fresh guinea-worm larvae, they can be stained in bulk by the same method. The larvae are placed in a small watch glass, in as small a quantity of distilled water as possible, and about 5 to 10 cc. of the fixative solution are added. The cover glass is warmed gently over a spirit lamp to about 60° to 70° C., and then allowed to cool to room temperature. The larvae are left in this solution for about 24 hours. Since the embryos will have settled down at the bottom by this time, the supernatant fixative solution may be removed by means of a pipette. The larvae are washed several times with distilled water, about 10 to 15 cc. of the stain are added, and this is allowed to act for about 36 to 48 hours. The stain is removed by means of a pipette and the larvae are washed several times with 2.0% formalin solution and are finally preserved either in 70% alcohol or in the 2.0% formalin solution.

Certain advantages of the method of staining described are:

1. The technique is simple and can be easily used even in field laboratories.
2. The larvae die in the fixative solution in a fully stretched condition.
3. Heating of the fixative fluid makes the larvae slightly adhesive to the slide and this prevents, to a certain extent, their moving out of the coverslip area while staining and mounting.
4. The specimens are very rarely overstained.
5. Since the stain is allowed to act on the larvae for a long time, it evaporates and concentrates very gradually giving varying depths of stain to the different parts of the larvae.

Acknowledgement is hereby made of the very valuable suggestions given by Lt.-Col. Glen Listin during this work. I am also greatly indebted to Dr. P. Parthasavathy, the Director of Health in Mysore, and to Dr. W. C. Sweet for the help and encouragement given by them.—V. N. Moorthy, *Office of the Director of Public Health, Mysore, Bangalore, India.*

COCKROACHES AS CARRIERS OF *GIARDIA* CYSTS

That cockroaches are capable of transmitting *Giardia* cysts has been indicated by a number of scattered observations. MacFie (1922, Ann. Trop. Med. and Parasit. 16: 441–448), Tejera, E. (1926, Rev. Soc. Argentina Biol. 2(4): 243–256), and Pessoa and Correa (1927, Rev. Biol. Hyg. 1: 90–93) fed cysts of human *Giardia* to cockroaches which were later recovered in the feces. One of the workers (Tejera) found the cysts as long as eight days after feeding.

The objects of these experiments were to determine the rate of passage of ingested materials through the alimentary tract of the roach and also to determine the length of time that cysts are retained in the roaches. This should give the length of time that roaches may act as carriers provided the cysts remain alive. No observations were made on the viability of the *Giardia* cysts. Human stools containing cysts of *Giardia lamblia* were used and the cysts concentrated by simple sedimentation or a combination of simple sedimentation and simple centrifugation methods.

Two genera and three species of cockroaches were used. *Periplaneta americana* were caught in Baltimore tunnels; *P. americana, P. brunnea,* and *Eurycotis floridana* were collected on the southwest coast of Florida. Upon collecting, fecal examinations were made and all were negative for *Giardia.* They were kept in separate clean cotton-plugged specimen bottles.

After starving for four to ten days to insure feeding, the cockroaches were fed the concentrated *Giardia* cysts. The animals were held in the left hand upon their backs and the material offered in a small mouthed pipette. The roaches ate the drops at the mouth of the pipette readily. In some cases nearly 1 cc. of the mate-

rial was ingested. They were given water but no food subsequent to the initial feeding. In making fecal examinations, the abdomen of the roach was gently pressed, thus forcing out a fresh droplet of fecal material. This was caught and examined in a wet smear preparation. This method of examination prevented the drying of the feces. Both nymphs and adults of the different species of roaches were used. Observations indicated that the passage of ingested material was similar in nymphs and adults.

The rate of passage was determined by feeding cockroaches liquified human feces stained with eosin to facilitate the following of the material. Observations were made by examining the contents of the various parts of the alimentary tract beginning at the posterior end. The location of the eosin-stained material could be seen often before opening the intestine but this was always confirmed by microscopic examination. Six *P. americana* were fed and autopsied after various intervals of time. After thirty minutes the crop was distended and slight traces of the material appeared in the anterior part of the mid-gut. In one hour the mid-gut showed considerable amounts and in two hours traces of the stained material were found in the anterior part of the colon. One roach contained none in the colon. After three hours the colon showed the stained material and at five hours contained a large quantity. Five *P. brunnea* and four *E. floridana* exhibited the same rate of passage as *P. americana*. Both species, however, showed traces of the eosin-stained material in the colon after two hours.

In determining the length of time cysts were retained, fourteen *P. americana* were fed *G. lamblia* cysts. Two autopsied five days later were positive. After seven days the feces of seven were examined and all were positive. On the eighth day two were autopsied and cysts found in both, whereas fecal examinations of three others were negative. Four were positive, one at autopsy, and two were negative on the ninth day. Three were still positive on the twelfth day. Ten *P. brunnea* were similarly fed. Three were kept alive as long as twelve days and showed *Giardia* cysts at the end of that period. The other seven were autopsied at intervals from the first to the tenth day and all were positive for cysts upon examination of the intestinal contents.

According to these experiments, *Giardia lamblia* cysts when eaten by cockroaches may reach the colon of *P. brunnea, E. floridana,* and in some individuals of *P. americana* in two hours. After three hours the cysts were present in the colonic contents of all three species. The cysts remained there as long as twelve days in both species of *Periplaneta.*—Martin D. Young, *Department of Protozoology, School of Hygiene and Public Health, Johns Hopkins University.*

A CURIOUS CASE OF ACCIDENTAL PARASITISM

Several years ago two students of mine examined for parasites a pigeon found dead on the University of Nebraska campus. A single, living trematode was found in the small intestine. This trematode was identified beyond all doubt as an immature specimen of *Clinostomum attenuatum* Cort, 1913. A prepared slide of the trematode shows it to agree in every detail with specimens from cysts in frogs (*Rana pipiens*). I observed the trematode at the time of collection when it was still alive. No live frogs had been in the building for several weeks. There can be no doubt that the trematode was actually living in the pigeon. But its size and degree of maturity plainly indicated that it had not been there long.

Clinostomum attenuatum is normally adult in bitterns and passes larval stages in snails and in frogs. The bittern acquires the worm by eating infected frogs. How could a pigeon, a strictly granivorous bird, possibly acquire such a parasite? The only clue which suggests itself in connection with this mystery is the fact that the pigeons on the campus live near Pharmacy Hall where many live frogs are used in physiology courses. It was near this building the pigeon was found. In some way the pigeon must have had access to the recently dissected body of a frog and from it eaten one of the loosely attached cysts which occur in the mesenteries, peritoneum and beneath the skin. These cysts doubtless attracted the attention of the

pigeon because of their resemblance to seeds. The circumstance illustrates how parasites may reach unnatural hosts by sheer accident. The death of the pigeon was not due to parasites.—H. W. MANTER, *University of Nebraska.*

A SHORT STUDY OF HUMAN PARASITISM IN THE MIDDLE NORTH

Through the kindness and cooperation of the Director of the International Grenfell Association Hospital at St. Anthony, Dr. Charles S. Curtis; the Secretary of the Department of Public Health and Welfare of the Government of Newfoundland, Dr. H. M. Mosdell; and in cooperation with the Government Pathologist, Dr. H. A. Ansley, the following preliminary survey of the parasitic protozoa of man was made possible. The study of some sixty individuals was completed during a three week stop-over at St. Anthony on a collecting trip for the Carnegie Museum, hence the small number of individuals studied. However, the following results were so striking that I felt this short note justified. Total number examined, 61; *Entamoeba coli,* 25; *Giardia intestinalis,* 13; *Amoeba* sp., 10; total infections, 39.

These results indicate a wide "spread" of conditions suitable to infection by intestinal protozoa; the patients ranged from Bonne Bay on the central west coast of Newfoundland to Nain, Labrador. They suggest that an introduction of the dysentery ameba into this area through tourist traffic, etc., might cause the people to be affected in epidemic proportions with the resulting disabilities, complications, and deaths. This condition is more of a problem in Newfoundland than in the States (where percentages of infections by these same organisms are also high, but not approaching those of this study), because of the great lack of propriety in the disposal of night soil, in the lack of screens on homes, and the enormous number of large house flies that contaminate every dwelling, fish-room, flakes, and dumping places of human excreta. I assume that the flies are the infective agents, although this must be studied further. The fact that such a high percentage of intestinal protozoa may be obtained through the study of only one smear (stained with iodine) from each stool indicates that with proper concentration methods, centrifugalization, etc., the percentage would have been greatly increased.—STANLEY TRUMAN BROOKS, *Curator, Section of Recent Invertebrates, Carnegie Museum, Pittsburgh, Pa.*

A QUANTITATIVE STUDY OF EGG PRODUCTION IN *TAENIA SAGINATA*

A review of the current literature on *Taenia saginata* indicates that there has been no actual count of ova in gravid segments. Welch (1875, Quart. Jr. Micr. Soc., N. Ser., 15: 325) stated that there are about 8800 ova present in a gravid segment or a total of 7,000,000 ova per worm. This figure is based on the assumption that each ovary contains 44 follicles and each follicle 200 ova. There is a mathematical error in this figure, since as Welch stated that there are 44 follicles "in each lateral half of the segment" there should be 88 follicles for the entire segment with a total egg count of 17,600 ova for the segment. Cobbold (Parasites, London, 1879) is quoted by several authors to the effect that the ova in a segment total 30,000. Reference to the original work shows this figure to be a simple assumption since Cobbold stated, "Any one of the links, 1250 in number, contains at one time, when perfect, fully 30,000 eggs." It was, therefore, with the view of clarifying this point that the writer undertook the problem of counting the ova in four gravid segments from four different specimens of *Taenia saginata* in the collection of the Division of Zoology of the National Institute of Health, Washington.

The technique was simple. The four terminal gravid segments, which had been preserved in 70 per cent alcohol, were transferred to a 10 per cent glycerin-alcohol mixture and carefully dissected to free the uteri. These organs were then gently teased to liberate the ova. The residual portions of the segments were preserved for examination as press preparations. The liberated ova were then counted by the drop method. One drop of the glycerin-alcohol was placed on a rough counting

chamber, a 2×3 inch slide etched to 25 mm², covered by a 1 inch cover slip, and the number of ova present recorded by means of a Veeder counter. To avoid duplication of counts, all ova resting on the lines to the right and above the central field were counted as belonging to that field. After all the eggs in the glycerin-alcohol were counted, the fragments of the segments were made into press preparations and the ova not released counted.

The egg counts and other data for the four segments examined are given below. Certain data could be ascertained for only three of the segments.

Segment	Catalogue No.	Date Collected	Source	Egg Count
1	10837	V/10/10	Wellesley, Mass.	117,855
2	11048	IV/13/13	St. Louis, Mo.	129,765
3	12404	VI/30/28	Hartford, Conn.	131,664
4	....	....	....	115,956
Mean				123,810

In these figures the variation from the mean of 123,810 for all four values is not greater than 6.3 per cent and not less than 4.09 per cent, and while the number of samples is not large the variation indicates that the figures are substantially indicative of actual egg production in *Taenia saginata.*

Cobbold (1875, Veterinarian, London, V. 48, 4. S. 572) has estimated experimentally that the average production of *Taenia saginata* is 400 gravid segments per month or 13.43 per day, a full grown cestode of this species containing, then, anywhere from 1000 to 2000 segments with the terminal fifth gravid at one time. The life of the adult *Taenia saginata* is reported by Leuckart (1886–1889, Die Parasiten des Menschen und die von ihnen herruhrenden Krankheiten) as from 1 year to 35 years (with a mean of 18 years). On this basis, the complete ova output for a single *Taenia saginata* was computed, with the following striking figures as the end result:

Ova per month	49,524,000
Ova per year	594,288,000
Ova present in gravid segments alone	37,143,000
Ova produced during life span of 18 years	10,698,184,000

With these figures in mind one can readily understand how such massive infestations with *Cysicercus bovis,* as reported by Stiles (Bull. 19, Bur. An. Ind., U. S. D. A., 1898) and Cobbold may occur. If cattle swallowed entire segments and all the ova developed into bladder worms, then, during the period of one year, were 1000 head of cattle to ingest only one proglottid apiece, the infestation of these animals would be sufficiently great to provide tapeworms enough to infest the entire population of the United States. Such an infestation would yield on microscopic examination 3 cysticerci per gram of muscle.

I am greatly indebted to Dr. Maurice C. Hall and the Division of Zoology in the National Institute of Health for the encouragement, scientific aid, space and equipment to carry out this investigation.—Mark M. Schapiro.

ELIMINATION OF AMOEBA FROM RATS WITH A HIGH PROTEIN DIET

Recently the writers described the development of a colony of rats that are free from trichomonad flagellates (1936, Jour. Parasit., 22: 408–409). This was accomplished by treating six rats with carbarsone and then rearing others from them in a room in which they were isolated from infected animals. Many of the rats in

this colony are still infected with giardias, hexamitas and amoebae. In an experiment just concluded we have found that the amoebae are apparently eliminated when diets high in protein are fed to the rats. Three types of diets were used as follows:

1. High carbohydrate diet		2. High protein diet		3. Liver diet	
Whole wheat flour	76.0	Casein	60.0	Dry beef liver	76.0
Casein	14.0	Dry yeast	15.0	Dry yeast	14.0
Butter	5.0	Whole wheat flour	12.0	Butter	5.0
Sodium chloride	1.0	Butter	8.0	Sodium chloride	1.0
Calcium carbonate	2.0	Sodium chloride	1.0	Calcium carbonate	2.0
Ferric citrate	2.0	Calcium carbonate	2.0	Ferric citrate	2.0
		Ferric citrate	2.0		

Ten rats were maintained on each of these three diets for a period of two weeks. They were then all killed and fresh cecal material examined microscopically. The entire contents of the cecum were also removed, stirred up in 60 cc. of Ringer's solution, kept warm in an incubator for 2 hours and then, after vigorous shaking to distribute evenly any organisms that might be present, a sample was counted in a haemocytometer. Amoebae were noted in every one of the 10 rats that were fed on a high carbohydrate diet, but no amoebae were found in any of the rats that were fed on the other two diets. We possess at present no method of cultivating amoebae from the rat, hence we are not absolutely certain that specimens were absent from the rats fed on high protein and liver diets. These experiments will be repeated when a satisfactory culture medium has been developed. The numbers of amoebae in the entire ceca of the carbohydrae-fed rats on the basis of haemocytometer counts were as follows: 10,350,000; 4,650,000; 2,850,000; 2,550,000; 1,500,000; 2,550,000; 6,300,000; 9,750,000; 2,850,000; 3,000,000. The average number per rat was 4,635,000. Our conclusion is that a diet high in carbohydrates creates a favorable environment for the amoebae in the cecum of the rat; whereas a diet high in protein but low in carbohydrates modifies the cecal contents to such a degree that amoebae are no longer able to live in this location.—Robert Hegner and Lydia Eskridge, *Department of Protozoology, the Johns Hopkins University School of Hygiene and Public Health.*

FASCIOLA GIGANTICA, A LIVER FLUKE OF CATTLE IN HAWAII, AND THE SNAIL, *FOSSARIA OLLULA*, ITS IMPORTANT INTERMEDIATE HOST

Specimens of flukes collected from livers of infested cattle on the island of Oahu have been determined by Mr. A. McIntosh and Dr. E. W. Price, of the Bureau of Animal Industry, Washington, D. C., as *Fasciola gigantica*. The writers have measured the eggs from 500 specimens of adult flukes collected on Oahu and these have been found to range from 156 μ to 197 μ in length, corresponding to the egg measurements given for *F. gigantica*.

Lymnaeid snails collected from liver fluke endemic areas on Oahu have been found naturally infested with the cercariae of *F. gigantica*. The identity of the cercariae has been ascertained by experimental feeding of the encysted cercariae to rabbits and the subsequent determination of the adult liver flukes recovered at necropsy from these rabbits. The snails from which these cercariae were derived and over 5,000 additional lymnaeid snails collected from streams and swamps in liver fluke endemic areas on the islands of Kauai, Oahu, and Hawaii have been identified as *Fossaria ollula* by Drs. J. P. E. Morrison and Paul Bartsch, of the U. S. National Museum, Washington, D. C. This snail has not been previously recorded as a vector of liver fluke and is apparently the important intermediate host of *F. gigantica* in Hawaii.—Joseph E. Alicata and Leonard E. Swanson, *Hawaii Agricultural Experiment Station, Honolulu, T. H.*

FIG. 1. a. *Fasciola gigantica* from liver of cattle in Hawaii; b. fresh-water snails, *Fossaria ollula* (snails slightly enlarged).

TWO *EIMERIA* FROM THE FLYING SQUIRREL, *GLAUCOMYS VOLENS*

In June, 1936, a flying squirrel, *Glaucomys volens,* was captured at Ames, Iowa. This squirrel, upon examination, proved to be infected with coccidia of the genus *Eimeria.* As far as can be ascertained, no coccidia have been reported from this genus of host.

The material collected was found to fall into two distinct groups when shape, size, and time of sporulaton was taken into consideration. First of these two groups (Fig. 1) contained coccidia which were characteristically cylindrical in shape and usually presented a rounded appearance at the poles. Some specimens were truncate at the ends. Oöcysts varied from 29.04–18.48 μ in length. In width they varied from 18.48–11.44 μ. Mean size was 23.83 × 13.69 μ. The spores were elliptical in outline and did not show a Stieda body. A sporocystic residual body was present. There was no micropyle present and no oöcystic residuum was observed. Sporulation took place in about 28 hours.

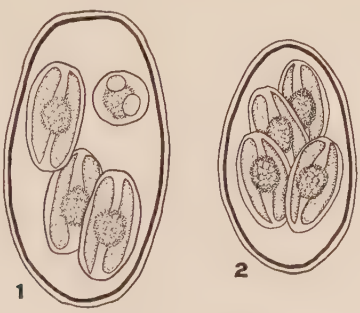

PLATE I

× 1850

FIG. 1. *Eimeria sciurorum* from *Glaucomys volens.*
FIG. 2. *Eimeria glaucomydis* n. sp. from *Glaucomys volens.*

Knipling and Becker (1935, Jour. Parasit., 21: 417–418) and Henry (1932, Univ. Calif. Pub. Zool., 37: 279–290) give summaries of the coccidia reported from squirrels. Most of the coccidia described from these hosts are referred to *Eimeria sciurorum* Galli-Valerio (1922, Centralbl. Bakt., II Abt., 56: 344–347).

The Eimeria found in the flying squirrel has some characters like *E. sciurorum* and so, for the present, at least, this coccidium is considered to belong to that species.

The oöcysts of the other coccidium (Fig. 2) isolated from the flying squirrel were elliptical in outline and were considerably smaller than those already discussed. They had a length range of 18.48–12.32 μ; width ranged from 13.20–10.56 μ. Mean size was 16.15 × 11.51 μ. Spores of this species were elliptical and like the former did not show a Stieda body. Sporocystic residual body was present but the oöcystic residual was absent. No micropyle was present. Sporulation time for this species was slightly less than 20 hours.

This coccidium appears to represent a distinct species and so the name *Eimeria glaucomydis* n. sp. is given to it.—Robert L. Roudabush, *Department of Zoology and Entomology, Iowa State College, Ames, Iowa.*

LEECHES INFESTING YOUNG WATERFOWL IN NORTHWEST IOWA

During the summer of 1936, in the course of a study of the American coot on Dan Green's Marsh in Clay County, Iowa, the writer found leeches (*Theromyzon occidentalis* (Verrill) parasitizing several young coots (*Fulica americana* Gmelin), pied-billed grebes (*Podilymbus podiceps* (L.)), and blue-winged teal ducklings (*Querquedula discors* (L.)). Identification of the leeches was verified by Dr. J. P. Moore, Department of Zoology, University of Pennsylvania.

The first infested coot noted was taken on July 28. This bird died approximately one-half hour after it was found. Shortly after the death of the coot a leech emerged from the nostrils. Upon dissection of the nasal cavity three additional leeches and a blood clot were found as obstructions in the air passages. Further detailed external and internal examination did not disclose any other death causing organisms. This coot was one week old and weighed 1.1 ounce. Three other coots were taken during the same day. The only external sign of parasitism was inflammation at the bases of the bills. In other ways, the birds appeared to be in very good condition. Laboratory examinations revealed two leeches and a clot of blood in the nasal cavity of the first bird, no infestation of the second coot, and one leech in the nostril of the third. These coots were about two weeks old and weighed 3, 4.5, and 5.5 ounces, respectively. On July 30, two more infested coots were taken. The bills of both of these specimens were inflamed at the base, and leeches were visible in the nasal cavities when the birds were held up to the light and pressure was applied at the bases of the bills. These coots were approximately two weeks old. The last infested coots were taken and banded on August 11 and 22. Leeches were visible when these birds were held up to the light and pressure was applied to the bases of the inflamed bills. These chicks were three weeks old and weighed 5 and 4.5 ounces, respectively. Observations were made on 17 other coots banded during the summer. They weighed from 6 to 19.5 ounces and were approximately three to seven weeks of age. None of these appeared to be parasitized.

Two twelve-day-old grebes were examined on August 8. The nasal cavities of both were infested with leeches. There were four leeches in the first grebe and three in the second. These birds weighed 3 and 2.2 ounces, respectively.

Six blue-winged teal ducklings were examined for parasites during the latter part of the summer. The first two were collected from a brood of seven on July 27. These ducklings were approximately one week old, were 7.5 inches in length, weighed 2.16 ounces and 2.2 ounces, respectively, and were approximately seven days of age. The first one was not parasitized, but the second had a single leech in its nasal cavity. Two other ducklings were taken from this same brood on

August 3. Each bird was 7.5 inches long, weighed 1.9 ounces, and was 12 days old. One duckling was parasitized by three leeches and the second by two leeches. The fifth and sixth ducklings were taken on August 21 and were apparently less than one week old. Only one of these was parasitized by a single leech in the usual manner. These birds each weighed one ounce and were 5.5 and 6 inches long, respectively. No mortality of young ducklings by leeches was noted during the summer's work.

There is a possibility that young birds when diving may become entangled in the dense growth of submerged vegetation present in most of the shallow lakes and marshes and drown as a result of their weakened condition resulting from parasitism by leeches. On July 10, a young coot with a long heavy string of algae and chara wrapped around its left leg was found dead. Evidently this bird had tired in its struggle with this water-soaked vegetation and drowned as a result There was no evidence of other causes of death. Thirteen other young coots were found dead during the summer. An examination of these did not disclose any external or internal evidences of death. It is possible that some of these might have suffered attacks by leeches, died, and then been deserted by the parasites.—CLARENCE A. SOOTER, *Department of Zoology, Iowa State College and Iowa State Conservation Commission, cooperating with the U. S. Biological Survey and the American Wildlife Institute.*

A SIMPLE SUBSTITUTE FOR ANTIFORMIN FOR PARASITOLOGICAL USES

A number of common parasitological technics call for the use of antiformin solution, but because of the instability of this solution, it is difficult in many climates to keep a supply on hand for occasional use. Several formulae for similar alkaline solutions containing free chlorine have been suggested, but one is suggested here which is simpler than most and very stable, and does not seem to have come to the notice of parasitologists generally.

This solution is prepared as follows. Into 1500 cc. of distilled water stir 400 grams of bleaching powder. In 500 cc. distilled water dissolve 440 grams of sal soda, or powdered sodium carbonate may be used in smaller quantity allowing for the difference in water of crystallization. When both solutions are cool, mix in a jar or flask and shake very thoroughly. Filter through an absorbent cotton pad in a Büchner funnel with a filter pump. These amounts make about one liter of solution with roughly 800 parts per million of free chlorine when made with the convenient stabilized bleaching powder now available, or of higher chlorine content if fresh ordinary powder is used. The exact amount of free chlorine may be easily determined by the standard method of titration in a dilute acid solution with N/100 sodium thiosulphate against the iodine freed by potassium iodide, using starch as an indicator. If stored in a bottle tightly closed with a slightly greased glass stopper, the chlorine content of the undiluted solution will remain unchanged for many months in any climate. The titrable alkalinity is about equal to a N/2 solution of sodium carbonate.

The solution is useful diluted to 30 per cent strength whenever sticky helminth eggs need to be freed from attached material as in the technic for isolating ascaris eggs from the soil as described by Spindler (1929, Amer. Jour. Hyg., 10: 157–164). It also has value when used in a 2 per cent solution for preserving eggs in feces (Scott, 1937, Jour. Parasit., 23: 109–112).—J. ALLEN SCOTT, *International Health Division of the Rockefeller Foundation and Department of Public Health of the Egyptian Government.*

THE EFFECT OF VARIOUS SOLUTIONS ON HELMINTH EGGS IN FECES

The preservation of helminth eggs in feces is an important problem in many types of work, since stools must frequently be examined at a considerable interval

after they are passed. The practical aspects of the problem have caused enough concern to reach expression in the literature, most frequently in connection with field investigations in hookworm disease. Lane (1919, Ind. Jour. Med. Res., Special Ind. Science Cong. Number, pp. 186–193; 1924, Trans. Roy. Soc. Trop. Med., 17: 407–436) early pointed out the difficulties of using levitation methods on stools more than 24 hours old, and concluded that the loss when diluted with water was not significant even at 28° C. The introduction of dilution counting of eggs eliminated from consideration any effects on the eggs other than those causing disintegration.

Chandler (1928, Ind. Jour. Med. Res., 15: 695–743) considered that stools in completely filled tins could be shipped without significant loss. Maplestone (1928, Ind. Med. Gazette, 63: 324–326) found that under identical conditions the loss was considerable, but was fairly consistent even at varying temperatures. Again he (1929, Ind. Jour. Med. Res., 16: 675–682) found that after the stools were in water the loss was considerable and attributes the divergence of his results from those of Lane to the predominantly vegetable diet of the people in his own series. As a preservative he recommended a 2 per cent solution of antiformin in which he found no loss up to 18 days at least. In a personal communication to the author he has since emphasized the need for a fresh antiformin solution or a similar solution of stable chlorine content.

During the organization of field studies in Egypt involving the examination of specimens from all parts of the country, it seemed desirable, if possible, to have the work done in one central laboratory. Before deciding on this plan it was necessary to make two determinations. The first was whether feces in partially filled tins at Egyptian summer temperatures would show a loss of eggs during the longest period between passage and delivery to the laboratory from the most distant point. The data regarding this point are not shown as they do not seem to be generally applicable in the form collected, but did show conclusively that under our conditions a 36-hour period could safely elapse. In the second place it was necessary to determine whether there would be a significant loss of eggs after stools were put into solution. The three protocols shown here are examples from more than twenty similar series made to test this point.

PROTOCOL 1. *Temperature range in laboratory 18° to 22° C.*

	Hookworm			*Ascaris*		
Days	Dist. Water	N/10 NaOH	2% Cl Sol.	Dist. Water	N/10 NaOH	2% Cl Sol.
0	21	24	18	311	307	388
1	21	23	15	414	322	385
2	18	20	24	575	282	394
4	16	17	18	375	278	319
5	16	16	17	310	312	299
6	15	23	16	364	297	284
7	13	14	17	386	227	332
9	14	22	15	369	373	312
11	19	16	16	391	326	313
13	13	16	12	406	309	307
15	15	11	13	376	306	269
18	13	13	16	346	272	333
26	16	11	11	264	270	300
32	15	13	17	279	342	302
47	9	11	15	291	271	298
79	1	8	5	249	262	228
Average 0–5 days	18	20	18	397	300	357
Average 26–47 days	13	12	14	278	294	300

The following solutions among others were used: 1. distilled water, 2. tap water with normal chlorination at water works, 3. NaCl in 0.7 per cent solution, 4. N/10 NaOH, 5. chlorine solution made as described by the author (Scott, 1937,

Jour. Parasit., 23: 109) and used in 2 per cent strength. Dilution was made in every case by adding 1 part feces to 14 parts solution by volume within 3 or 4 hours of passage of stool. In protocols 2 and 3 the suspensions were strained and sedimented and the supernatant fluid drawn off to increase the size of the counts. The first counts were made within one hour of dilution. All observations are expressed in terms of the average number of eggs per 0.015 cc. of feces, i.e., the standard amount on a small Stoll slide, but are based on the counts of 3 slides from a single flask in protocol 1, and on 3 slides from each of 3 flasks in protocols 2 and 3.

The data show that during the first three days in these solutions, no species of eggs showed a decrease that was significant in light of the variability inherent in the method of counting. In most cases the decrease was not appreciable during the first five days, but after five days the counts sometimes began to decrease. It is surprising that, as shown in protocol 1, the supposedly frail hookworm eggs did not show any great decrease up to 32 days, even in distilled water. This protocol was typical of all similar series except that in chlorine solution

PROTOCOL 2. *Temperature: 26–30° C.*

Schistosoma mansoni

Days	Tap Water	0.7% NaCl	N/10 NaOH	2% Cl Sol.
0	34	34	20	20
1	30	36	29	30
2	31	35	14	30
3	24	50	25	46
4	14	51	26	36
6	27	45	19	21
9	23	46	17	23
13	15	28	15	21
27	24	29	11	34
36	17	31	6	27
49	30	15	5	43
126	17	..	2	21
434	22	35	0	5
Average 0–4 days	27	41	23	32
Average 27–49 days	24	25	7	35

Percentage of count represented by empty egg shells

Average 0–4 days	14	1	35	38
Average 27–49	53	24	63	37

PROTOCOL 3. *Temperature: 26–30° C.*

Schistosoma mansoni

Days	Tap Water	0.7% NaCl	N/10 NaOH	2% Cl Sol.
0	75	74	81	77
1	59	71	53	52
2	53	96	57	38
3	50	67	67	39
5	66	75	53	45
8	55	68	35	41
12	50	42	38	38
26	35	61	25	91
35	35	38	37	35
48	55	28	61	30
125	..	44	22	38
433	50	49	40	45
Average 0–5 days	61	77	62	50
Average 26–48 days	42	42	41	52

Percentage of count represented by empty egg shells

Average 0–5 days	10	7	28	24
Average 26–48	54	23	50	34

there was usually no loss during the first two months at least. In sodium carbonate solution of the same titrable alkalinity hookworm eggs were usually not preserved well. It is also surprising that the reputedly resistant ascaris eggs (all of which were fertilized) were beginning to decrease toward the end of all the series in protocol 1.

Protocols 2 and 3 show that except for one case which was not entirely typical, there was relatively little decrease in the numbers of eggs of *S. mansoni.* Again the chlorine solution usually seemed to be the best solution for preservation over a period of weeks. For preserving the specimens for the few days usually necessary in keeping a laboratory in step with a collecting crew, any of these solutions seem to be satisfactory. Hoffman (1934, Puerto Rico Jour. Pub. Health and Trop. Med., 9: 283–298) stated that NaOH seemed to have an adverse effect

on *S. mansoni* ova but has since told the author he was referring to the killing of the miracidia. In our series the viability of the ova was not considered to be of importance. In fact we counted all identifiable egg shells, the proportions of empty shells in the counts being shown for the averages in protocols 2 and 3. It is evident that the percentage of empty shells was higher at the beginning in NaOH and chlorine solution than in water or saline. Evidently some stimulus to hatching or bursting is present in these solutions but how it acts is not clear. No reason can be given why hatching did not occur in water unless it be due to the chlorine of the drinking water or the residual alkali not removed from the flasks by scrubbing in hot water, or to the fact that dilution was not great enough to change the osmotic tension sufficiently. The proportion of empty shells does not seem to increase during the first 5 days in any of these or other parallel series. In the period 26 to 49 days the proportions of shells is signficantly larger than for the early period except in the case of chlorine solution.

Eggs of *S. haematobium* in these solutions behaved in the same way as those of *S. mansoni*.—J. Allen Scott, *International Health Division of the Rockefeller Foundation and Department of Public Health of the Egyptian Government.*

A METHOD FOR PRESERVING AND SHIPPING SMEARS OF PROTOZOA

During the past few years, at the University of Pennsylvania and Yale University, the writer has secured collections of opalinid ciliate protozoons, which live in the rectum of frogs and toads, from a number of distant localities, including Peiping, Nanking, Foochow, Hong-Kong, Colombo, Panama City, the City of São Paulo, and Cairo, and has used the following method for preserving and shipping the smears of these protozoons. Essentially, the method consists of the use of paper rings as washers between the cover glass smears as they are placed in the vial (Fig. 1), in order to avoid crushing the animals. For the opalinids the method is as given below. It may, however, also be employed for handling smears of other kinds. After the smears are fixed, washed, and dehydrated until they are in 70% alcohol, they are transferred to a vial.

Two paper rings are first placed at the bottom of the vial partially filled with 70% alcohol. Next one smeared cover slip is placed with the smeared side down onto the two rings. Another paper ring is inserted in the vial followed by the

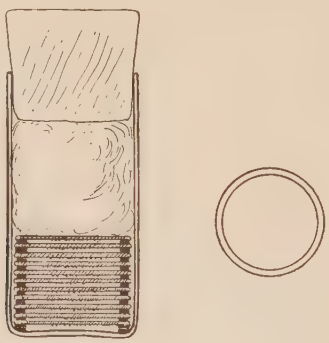

Figure 1. a. Diagrammatic longitudinal section of vial containing the smeared cover glasses with paper rings as washers. b. The paper ring.

second smeared cover slip, also with the smeared side down. The process is repeated until all the smeared covers from a single host have been packed into the vial, which is then filled with more 70% alcohol. A label bearing the name of the

host and locality is then placed inside the vial, before a wad of cotton is added, which prevents displacement of the contents in transportation. The vial is now corked tightly and finally sealed with paraffin.

In case it is desired to put material from different sources in one vial, a label may be placed on top of a series of covers, then a thin cotton plug, followed by rings and covers from the next source or host. Other additions may be made until the vial is comfortably filled with space left for the final plug of cotton and the stopper. It may be desirable to store these vials containing the smears in large bottles containing 70% alcohol in order to prevent evaporation of the alcohol in the vials. In such a case, no corks for the vials are necessary. The cover glasses used are No. 1, round, and are 22 mm. in diameter. The vials have an inside diameter of 24 mm. and a height of 50 mm., while the paper rings have an outside diameter of 22 mm. and an inside diameter of 19 mm. and are made of fairly thick cardboard. Each vial of the size as described has a capacity of 20–25 smears. Vials of greater height may be used if more smears are to be carried in each vial. The paper rings and the vials of the special size may be had from the Macalaster Bicknell Co., 412 Temple Street, New Haven, Connecticut.—Tze-Tuan Chen, *Osborn Zoological Laboratory, Yale University.*

AN EXPERIMENTAL INFECTION OF *PLAGIORCHIS MURIS* IN MAN

Numbers of *Stagnicola emarginata angulata,* collected at the University of Michigan Biological Station at Douglas Lake, infected with a common Xiphidiocercaria have been found to have numerous metacercariae of the same species in the sporocysts. Dollfus (1925, Ann. Parasitol., 3: 185–205) translated a paper by Tanabe (1922, Okayama Izakkai Zasshi, 47–58) who reported a similar precocious development in *Plagiorchis muris* in the snail *Lymnea pervia.* Mice were experimentally infected by this author with these cysts and wild rodents were found to be naturally infected. Cercariae that emerged from the snail were found to penetrate into insect naiads. Bats were suggested as the probable definitive host for the metacercariae that develop in the insects.

In an attempt to obtain adults of the metacercariae found in *Stagnicola emarginata angulata,* the cysts were fed to rats, mice and pigeons. Details of these experiments are to be published in a later paper. At present it is sufficient to state that normal development took place in all three species of experimental hosts and the adults were identified as *Plagiorchis muris* Tanabe, 1922. Natural infections of this trematode have been found in the Douglas Lake region in a wide variety of birds, i.e., robin, nighthawk, herring gull and spotted sandpiper. An infection has never been found in bats.

This remarkable lack of specificity is very unusual. Such a wide range of hosts suggested that this species might even infect man. Examinations of animals with heavy infections showed no evidence of pathological conditions. It was therefore considered safe to attempt a human infection. One hundred mature cysts were dissected from a snail, washed and ingested in physiological salt solution. One week later 150 more metacercariae were ingested and stool examinations were started. A large number of cysts were taken because it was feared that the loss of numerous metacercariae and the possible reduction in egg production in the abnormal host would make it difficult to detect the presence of the adult flukes. On the ninth day after the initial infection the first eggs (Fig. 1) were found. These measure 0.038×0.019 mm. and are in the morula stage. After the twelfth day, when the egg counts were reaching considerable proportions and it seemed probable that the infection was going to have a more or less normal span, a regular routine examination was begun. This consisted of the daily examination of three smears, using the Stoll egg counting method, and using the average in calculating the number of eggs per stool.

The complete history of the infection is shown in the accompanying graph (Fig. 2). It will be noted that a high point of 39,300 eggs is reached on the four-

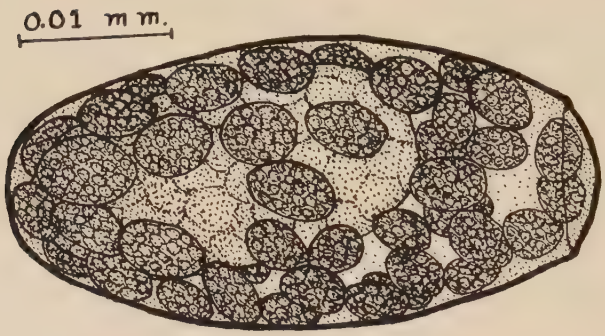

FIG. 1. Egg of *Plagiorchis muris.*

teenth day after the initial infection. This is followed by a falling off in egg production for two consecutive days, then the number of eggs increased again. Late in the seventeenth day the infected individual suffered from a severe attack of indigestion, as a result of injudicious eating. The examination 38 hours later showed a marked decrease in the egg production, from 66,790 to 3,900 per day. It is impossible to state definitely that this decrease was caused by the digestive disturbance, but it certainly is suggestive. In two more days the egg production ascended to a new high of 74,700 per day. It will be noted that this high point was reached just fourteen days after the second infection. The number of eggs recovered from the second infection indicates that no immunity was produced by

FIG. 2. Egg production of *Plagiorchis muris* in man, experimental infection.

the initial infection. From this point until the thirty-seventh day of the experiment the egg production tended to decrease. It might be well to state that most of this latter period, from the twenty-first day to the thirty-first day, inclusive, consisted of a cross country visiting tour. Possibly this may account for some of the irregularities in the counts during this period.

Throughout the entire experiment the stools were normal and regular. The eggs in the feces were the only evidence of the infection. No symptoms, due to the presence of the trematodes, of a pathological nature were detected. The number of eggs produced during the experiment was approximately 605,700, an average of 20,883 per day. The number of trematodes present is not known but it is believed that a high percentage of the metacercariae were able to establish themselves. One reason for making this statement is that an 8-day-old trematode

of this species, in other experimental animals, has only about 500–700 eggs in the entire length of the uterus.

The experiment has no significance from the standpoint of public health but it is believed to be the only record of a member of the family Plagiorchiidae infecting man.—Donald B. McMullen, *Biology Department, Monmouth College.*

FEEDING EXPERIMENTS ON CATS WITH *GNATHOSTOMA SPINIGERUM* LARVAE OBTAINED FROM THE SECOND INTERMEDIATE HOST

In our two previous papers (Prommas and Daengsvang, 1933, Jour. Parasit., 19: 287–292, and 1936, Jour. Parasit., 22: 180–186) it was reported that cyclops and fresh-water fish (*Clarias batrachus* Linnaeus) were found to be respectively the first and second intermediate hosts of *Gnathostoma spinigerum*. The present experiments deal first with attempts to find other kinds of fresh-water fish that may also serve as the second intermediate host of the parasite, and second with efforts to infect the definite host with parasitic larvae obtained from the second intermediate host.

Young fresh-water fish, *Ophicephalus striatus* (Block) used in the experiment were kindly supplied by the Department of Fisheries of the Government. Many of them were sacrificed and subjected to careful examination for natural gnathostome infection and none was positive. Four of the remaining fish were placed each in a separate rectangular museum jar containing sufficient water and allowed to feed on various numbers of infected cyclops harboring *G. spinigerum* larvae of different ages and numbers. Fish No. 1 fed with twelve cyclops containing a total of 18 larvae sixteen days of age was killed twenty days after feeding and on examination revealed two grown living non-encysted larvae in the body muscle. Fish No. 2 was killed thirty-seven days after feeding with eighteen cyclops containing altogether 29 sixteen-days-old larvae and was found to harbor 4, 3 and 2 grown living non-encysted larvae in the intestinal wall, stomach wall, and body muscle respectively. Fish No. 3 fed with twenty-eight cyclops containing a total of 43 eighteen-days-old larvae, died 11 days after by jumping from the jar and revealed one grown living larva in the body muscle and one similar larva in the liver. Fish No. 4 was killed sixty-one days after feeding with fifteen cyclops containing a total of 29 twenty-one-days-old larvae and revealed one encysted larva in the body muscle and two grown living non-encysted larvae in the liver. All larvae found in the four experimental fish had attained the size and morphology identical with those found in *C. batrachus* Linnaeus, previously described by us.

Three cats (Nos. 23, 36 and 38), which had been kept in the laboratory over a year and definitely proven negative for gnathostome infection by frequent careful fecal examinations, were used in the experiment. Cat No. 23 was fed with two grown non-encysted *G. spinigerum* larvae obtained from the liver of fish No. 4 (*O. striatus*), cat No. 36 with four grown non-encysted larvae from the body muscle of *C. batrachus* Linnaeus, and cat No. 38 with nine grown non-encysted larvae from fish No. 2 (*O. striatus*). The feeding was done in a natural way. The animals were kept in the same cage and their food and drink were under strict control; their stools were frequently subjected to microscopic examination for gnathostome ova. Cat No. 23, 180 days after feeding, was found to be negative for the parasite by both fecal and post-mortem examinations. Cat No. 36 became positive by fecal examination 223 days after the experiment and was sacrificed 39 days later and autopsy showed a firm oval nodule ($2.5 \times 1 \times 0.7$ cm.) in the gastric wall at the middle portion of the lesser curvature, projecting into the stomach cavity. It was covered with apparently normal mucosa and presented a small central opening 2 mm. in diameter; the corresponding serosa was hyperemic. On sectioning the nodule there was found an irregular hyperemic cavity walled by thick gray firm tissue and containing two adult males and one adult female of *G. spinigerum*. Cat No. 38 showed the parasitic eggs in its stools 198 days after

feeding, was killed 66 days later and on autopsy revealed a firm, somewhat rounded tumor (2.6×2×1 cm.) in the stomach wall on the greater curvature near the pylorus and protruding outward (Figs. 1 and 2). The covering mucosa

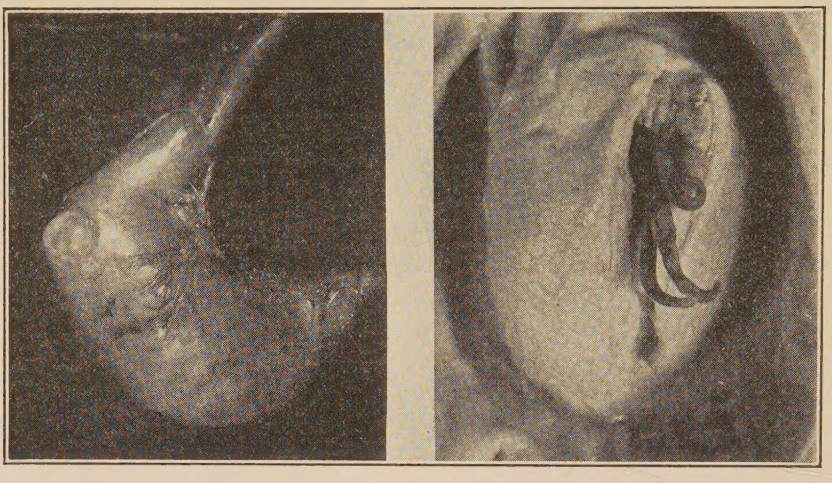

FIG. 1. (Left) Stomach of cat No. 38, showing *Gnathostome* tumor protruding outward.

FIG. 2. (Right) Stomach of cat No. 38 after dissection, showing three protruding adult *G. spinigerum*.

was apparently normal and showed a small opening near the lower edge of the tumor; the covering serosa was smooth and marked with a few small yellowish-gray opaque areas. The tumor on section was made up of gray firm dense tissue in which was an irregular hyperemic cavity filled with 4 adult males and 4 adult females of *G. spinigerum*.

The facts obtained from our present and previous studies have clearly explained the life cycle of *G. spinigerm.* The adult female parasite after fertilization lays eggs which are passed out with stools of the definite host and hatch in water into active motile sheathed larvae. The time for hatching varies according to temperature, taking about ten days in warm condition and longer in cold atmosphere. The sheathed larvae when kept longer in water show no further change in size and structure and die in a few days, unless they are ingested by cyclops. In the body of the latter they increase in size, undergo metamorphosis and develop to a form that is furnished with a cephalic bulb ornamented with four transverse rows of single-pointed spines, a pair of trilobate lips, two pairs of contractile cervical sacs and a rudimentary alimentary system. These larvae are not able to infect the definite host but are able to grow in the body of fresh-water fish, *C. batrachus* (Linnaeus) and *O. striatus* (Block) to a larger larval form which shows in addition a cellular column, probably a rudimentary reproductive organ, and finally becomes encysted; the cyst wall is thin, fibrous and apparently produced by tissue reaction of the fish. Such larvae not yet encysted when fed to the cat, the definite host, develop in the stomach wall to adult worms causing definite tumors and pass eggs about six months after ingestion. Probably man, the unsuitable host of the parasite, gets the parasitic infection in the same way as cats.

The authors are indebted to the Department of Fisheries of the Government for supplying fish for the experiment and to Dr. K. Ketudasna for kindly taking the photographs illustrating this paper.—CHALERM PROMMAS AND SVASTI DAENGSVANG, *Department of Pathology, Faculty of Medicine, Chulalongkorn University, Bangkok, Siam.*

REVIEWS

THE INTERNAL PARASITES AND PARASITIC DISEASES OF SHEEP. Their Treatment and Control. By I. Clunies Ross and H. McL. Gordon. 238 pp. 46 pls. Angus and Robertson Ltd., Sydney, Australia.

This book includes only the helminth parasites of sheep and emphasizes their life-history, pathogenicity, treatment, and control. Species that are of economic importance in Australia are particularly stressed. Distribution and control are discussed chiefly in relation to Australian conditions. While this of course limits somewhat the usefulness of the book for sheep raisers in other countries it adds greatly to its interests to specialists in helminthology. For example the series of maps showing the distribution of a number of the most important species in Australia brings together a mass of information not easily available elsewhere. A quotation from the introduction gives an idea of the method used in presenting the material: "The manner of presentation is designed to assist both the scientist and the layman to find conveniently those aspects of a given problem of special interest to them. To this end the macroscopic and microscopic characters of a group or of individual parasites, which are of purely scientific interest, are first described. This is followed in the case of those parasites of major economic importance by a separate section containing a detailed description of their life-histories and methods of treatment and control. This material, which it is hoped will be easily followed by stock owners and laymen generally, is set more widely than that of scientific interest only. . . ." In general the book is successful in accomplishing these objectives and will be of interest and value to those interested in the parasites of sheep either from the practical or scientific viewpoints.—W. W. CORT.

STUDIES ON NORTH AMERICAN CERCARIAE. By Edwin Lynn Miller. Illinois Biological Monographs, Vol. 14, no. 2, 125 pp., 108 figs. (University of Illinois Bulletin, Vol. 33, No. 35, April 28, 1936).

This monograph includes data on 23 different species of cercariae which were studied by the author, four of which are considered as new. New details of structure are added on the 19 previously described forms, the majority of which were described by the author in a paper published in 1935. The morphological studies were made almost entirely on living material and the details of structure are well worked out. In the analysis of the relationship of the cercariae, it seems to the reviewer, that too little attention is paid to the recent work on life cycles as an aid in assigning cercarial groups to their proper systematic position. For example in the light of present knowledge of life cycles there seems little need for the retention of the old larval classification for the forked-tailed cercariae. Also the author goes directly contrary to the opinion of most workers on the life cycles of digenetic trematodes, which is based on very extensive evidence obtained chiefly in the last few years, when he accepts the view that cercariae very similar in structure may have different kinds of development and that cercariae very different morphologically may have similar courses of development. The chief value of this monograph will be the aid it will give in further studies on the life cycles of North American trematodes.—W. W. CORT.

STUDIES ON THE MORPHOLOGY AND LIFE HISTORY OF NEMATODES OF THE GENUS *Spironoura*. By John Gilman Mackin. Illinois Biological Monographs, Vol. 14, No. 3, 64 pp., 69 figs. (University of Illinois Bulletin, Vol. 33, No. 52, August 25, 1936).

The material in this monograph covers three separate but related topics. An extensive description is given of the anatomy of *Spironoura chelydra* (Harwood) 1932 with special attention to the histology of the arcade system, the entire digestive tract, the genital systems, and the supportive structures of the head. A revised diagnosis of the genus *Spironoura Leidy,* 1856, the descriptions of two

new species, *S. wardi* and *S. concinnae,* and a key to the North American species of the genus are included. The author also includes an analysis of measurements of *S. chelydrae* in relation to growth and variations to test the value of formulae designed to aid in the separation of species of nematodes. He concludes that nematodes formulae are practically valueless as a basis for separating species and are a source of much confusion.—W. W. Cort.

The Life History of *Cotylophoron cotylophorum,* a Trematode from Ruminants. Illinois Biological Monographs, Vol. 14, No. 4, 119 pp., 81 figs. (University of Illinois Bulletin, Vol. 34, No. 9, September 29, 1936).

This monograph gives a detailed description of the development of the various stages of the life cycle of this important amphistome of ruminants from an abundance of material obtained by experimental infections of both intermediate and definitive hosts. The time involved in the development of the different stages is also worked out. The section on the egg and structure and development of the miracidium is especially detailed covering 26 pages. A comparison of the number and arrangement of the ciliated epidermal cells from the descriptions of 18 different species of miracidia leads the author to the conclusion that they are of taxonomic value. The development of the mother sporocyst is traced in detail from the entrance of the miracidia into the snail intermediate host up to the full development of the rediae. Each mother sporocyst produces 9 rediae and each redia about 25 cercariae. Second generation rediae were observed only in one case. Further stages of development are traced with the same care and detail. This study adds much information on the development of this species and is an important contribution to our knowledge of the life cycles of the amphistomes.—W. W. Cort.

ERRATA

Errata in Research Note by Sherwin F. Wood and Fae D. Wood, "Occurrence of Haematozoa in Some California Coldblooded Vertebrates," Journal of Parasitology, vol. 22, no. 5, pp. 518–520.

Page 519, paragraph 5, line 2, "strain" should read "stain"
Page 519, paragraph 6, line 3, "strains" should read "stains"
Page 519, paragraph 6, line 4, "strains" should read "stains"
Page 519, paragraph 6, line 5, "lighter-straining" should read "lighter-staining"
Page 520, paragraph 2, line 4, "straining" should read "staining"
Page 520, paragraph 2, line 5, "strains" should read "stains"
Page 520, paragraph 3, line 2, "straining" should read "staining"
Page 520, paragraph 6, line 2, "Conty" should read "County"
Page 520, paragraph 6, lines 18, 19, "tesselatus" should read "tessellatus"

Dates of issue for numbers of volume 22 (1936):

No. 1, February 27
No. 2, April 23
No. 3, June 22
No. 4, August 21
No. 5, October 22
No. 6, December 16